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VRIJE UNIVERSITEIT
MECHANISTIC MODELS TO EXPLORE COMBINED
EFFECTS OF
TOXIC CHEMICALS AND NATURAL STRESSING
FACTORS:
CASE STUDY ON SPRINGTAILS

ACADEMISCH PROEFSCHRIFT

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de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. F.A. van der Duyn Schouten,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Aard- en Levenswetenschappen
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De Boelelaan 1105

door

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This thesis is dedicated to T. A. Nega

Nothing is as real as a dream. The world can change around you,
but your dream will not. Responsibilities need not erase it. Duties
need not obscure it. Because the dream is within you, no one can
take it away. – Unknown

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For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own.

Chapter 1

Introduction

1.1 Ecological risk assessment of chemicals

There is a growing awareness that economic development has had, and continues to have, a major impact upon the natural environment. On the other hand, the need for such growth to improve living standards in the society is also well recognized, and generally highly appreciated. To maintain a balance between the environment and the economy, and address this economic growth vs. environmental problems paradox, different tools have been developed and used. The ultimate goal of these tools is to facilitate environmentally-oriented decision making and integrate the environmental dimension in the economy. One such tool is ecological risk assessment (ERA). According to US EPA's *Framework for Ecological Risk Assessment*, ERA is defined as a process that evaluates the likelihood that adverse ecological effects may occur, or are occurring, as a result of exposure to one or more stressors [1].

Consistent with this general framework of the ERA process, chemical risk assessment procedures have also been developed. In recent years, this process became an essential legal requirement in most countries.

The overall aim of these regulatory risk assessments is to quantify the likelihood that chemicals may cause undesirable impacts on ecosystems and human health. This information allows and facilitates an informed decision making in controlling the production, use and release of chemical substances. In general, the process involves three phases: problem formulation, analysis, and risk characterization [2] and provides a qualitative and quantitative description of the nature of the potential adverse effects, the probability that these effects will occur, as well as their extent [3–5]. In practice, a quantification of probabilities is extremely rare.

During the problem formulation phase, the decision is made which aspects of the environment should be selected for evaluation; in other words, assessment end-points are defined. In the analysis phase, assessors evaluate the exposure to stressors and the relationship(s) between stressor levels and effects on selected end-points. This phase connects the problem formulation with the risk characterization and examines the two primary components of risk, exposure and effects, and their relationships. In the third phase, the risk characterization, assessors estimate risk through integration of exposure and stressor-response profiles, describe risk by discussing lines of evidence and determining ecological adversity, and prepare a report [2]. The predicted environmental concentration (PEC) and predicted no effect concentration

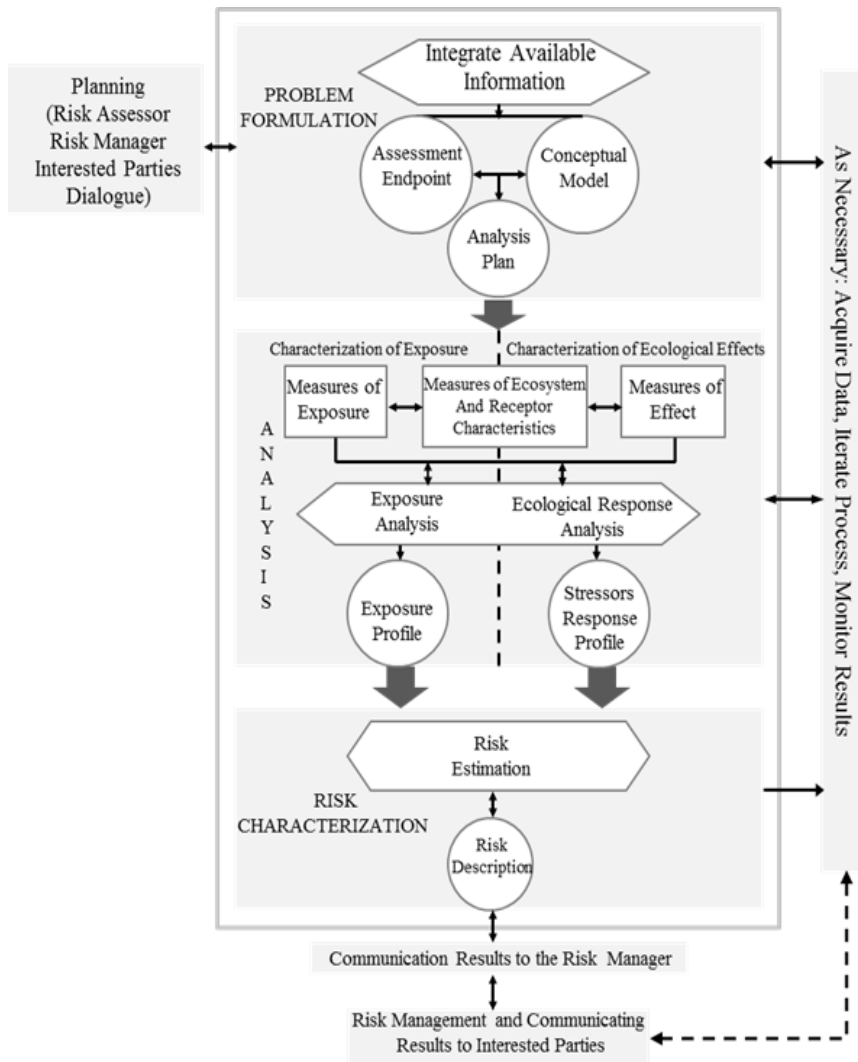


Figure 1.1: The ecological risk assessment framework, with an expanded view of each phase. Within each phase, rectangles designate inputs, hexagons indicate actions, and circles represent outputs. Adapted from [2].

(PNEC) are used to represent exposure and effects respectively. The PEC represents the level of the contaminant in the environment, while the PNEC is the concentration below which the chemical is expected not to influence the assessment endpoints as defined during the problem formulation.

1.2 Extrapolation of effects

As mentioned above, potential adverse effects of chemicals are judged by comparing PNEC against PEC. The PEC can be estimated using either fate and transport models or by direct measurements of chemicals concentrations in the environment. The PNEC is usually derived from bioassays quantifying effects on individual life-history traits: survival, growth or reproduction. However, ERA aims at protecting populations or whole communities rather than individuals [6–10]. The important question in the estimation of PNEC is, therefore, how to make the connection between measurements made on individual organisms and the higher-level assessment endpoints. To address this issue, risk assessment procedures typically include safety (or uncertainty) factors in order to ensure conservative estimates of environmental concentrations that minimize risks posed by chemicals to organisms in the natural environment [11]. Despite their wide application, the ability of these uncertainty factors for ex-

trapolating effects has been questioned by different authors [11, 12]. Among others, lack of transparency and neglecting potentially important variables in the key step between individual-level responses and population-level impacts are the main limitations of these methods [11]. The selection of uncertainty factors is also arbitrary and lacks any scientific approach; which makes their accuracy difficult to assess.

To overcome this limitation of the current approach to extrapolation from individual-level bioassays to higher-level risk assessment, population models are advocated [11, 13–15] and, an interest has developed to integrate population models in the risk assessment process. The interest to shift from studying effects of toxicants on individuals to populations is also occurring with increasing emphasis on population-level assessments by regulatory agencies such as the US Environmental Protection Agency [2]. The intent of these models is to extrapolate organism-level effects observed in laboratory to population-level impacts that may happen in the field.

1.3 Key scientific issues in developing population models

Although the potential for adding value to ecological risk assessment by applying population models is recognized, there are issues that should be addressed when developing these models. One important

issue is the need for parameterization of the models [11]. The parameterization process requires abundant quantitative information about the values that model parameters take under different exposure scenarios, and this information should be derived from laboratory-based bioassays and experiments. Unfortunately, these toxicity tests are usually conducted under constant environmental conditions, which helps isolating effects of a chemical but neglects potential influences of all other environmental factors. What is more, the experimental conditions are presumably optimal for the test organisms. However, in their natural environment, organisms rarely experience optimal or constant conditions. On the contrary, they have to cope with either suboptimal conditions or even severe environmental stress for most of their life spans [17, 18].

The influence of these added environmental stressors on the effects of chemical contaminants in comparison to laboratory tests performed under controlled and optimal conditions is discussed in several papers. In a recent meta-analysis, Laskowski et al. [19] reviewed and analyzed interactions between a range of natural environmental factors and toxicants in animals. Holmstrup et al [17] evaluated the interactions between effects of natural and chemical (anthropogenic) stressors. In their review, the authors found that all of the natural stressors they analyzed showed an interaction with a variety of envi-

ronmental pollutants, and more than 50% of the studies reported a synergistic interaction.

Beside effects of natural physico-chemical stressors, interactions between toxicants and density-dependence and their influence on population dynamics have gained interest among ecotoxicologists more recently [20–27]. Thus, parameterization of population models using vital rates obtained from standard bioassays limits the applicability of these models, making the extrapolation to, and interpretation at, the population level uncertain. Therefore, understanding and including intra-population and extra-population ecological factors that can modify effects of toxicants at individual and population levels is a key step in developing tools for extrapolating the results of laboratory toxicity tests to populations living in natural environments.

1.4 Research questions and study objectives

The aim of this thesis is to explore and understand how environmental and other external factors affect soil invertebrate populations exposed to toxicants, and to develop models that could be used to predict and extrapolate the combined effects of toxicants and environmental factors from individual-based laboratory experiment to realistic field conditions.

The following research questions have been formulated:

- does impact on and recovery of populations, which are exposed to toxicants, depend on ecological factors such as life-history characteristics, population structure, density-dependence, and temporal environmental fluctuations (variability)?

If so,

- How can we integrate these factors to extrapolate effects of toxicants from individual to population, and from certain environmental settings to different ones?
- How does sensitivity at the individual level mechanistically link to impacts on populations?

To answer these research questions and address the objectives, I develop and present models that can predict the combined effects of toxicants and environmental stressors on populations exposed to chemicals. The conceptual framework of the models comprise two basic elements: an individual-level effects model and a population model to extrapolate individual-level effects to population. Both empirical and mechanism-based models are employed to model effects at the individual-level. The mechanism-based model is briefly introduced in Section 1.5. Models for the extrapolation of individual-level effects are based on the classical structured population models, which briefly introduced in Section 1.6. The model development is structured based

on the logical principles of Good Modelling Practice GMP and follows cyclical and iterative procedures. Beside the models that address the main research question posed in the thesis, I also develop and present a modeling technique that can help the design of short-term ecotoxicological tests for the direct interpretation of individual-level effects to population.

1.5 Energetics based TK/TD individual-level effect model

Individual-level effects are usually estimated from the classical concentration-effect (or dose-response) models. These models are empirical in nature and derived by fitting bioassay data. However, typical bioassays do not take into account the factor of time and other environmental components that can influence effects of chemical at the individual-level [28]. These limitations can be overcome by developing individual-level mechanistic effect models.

In this study, I developed and parameterized an energetic-based model for the mechanistic interpretation of individual-level effects of chemicals. The mechanistic individual-level model I applied in this study is based on the Dynamic Energy Budget (DEB) theory. DEB is the most comprehensive and best-tested theory for metabolic organization and captures the processes of development, growth, maintenance, reproduction and ageing for any kind of organism throughout its life-

cycle [29]. A coherent set of assumptions leads to the basic DEB animal model, which is supported by empirical facts [30]. These features make DEB a general and comprehensive framework in different model studies and with practical applications in ecotoxicology [31–33]. One special feature of the theory is the κ - rule; which assumes that, a constant fraction κ is allocated to growth and somatic maintenance (the soma), and the remainder, $1 - \kappa$ to maturity maintenance, maturation and reproduction. This implies that growth and reproduction do not compete for resources directly [29]. Figure 1.2 shows the schematic description of energy allocation in an animal in DEB theory. The DEB model I applied in this study is a simplified version of the standard DEB animal model as presented by Sousa et al. [30], known as DEBkiss [34]. The main simplification of the DEBkiss model is that it does not have a reserve compartment and that maturity is not considered as a state variable [34].

In the DEB context, the effects of toxicants on vital rates of an organism result from changes in one or more of the metabolic processes in Figure 1.2 [33]. The severity of the effect on the metabolic process is directly related to the concentration of the toxicant inside the organism. A first-order one compartment toxicokinetics (TK) model is applied to link measured environmental concentrations to internal concentrations. The core assumption for the TK model is that the up-

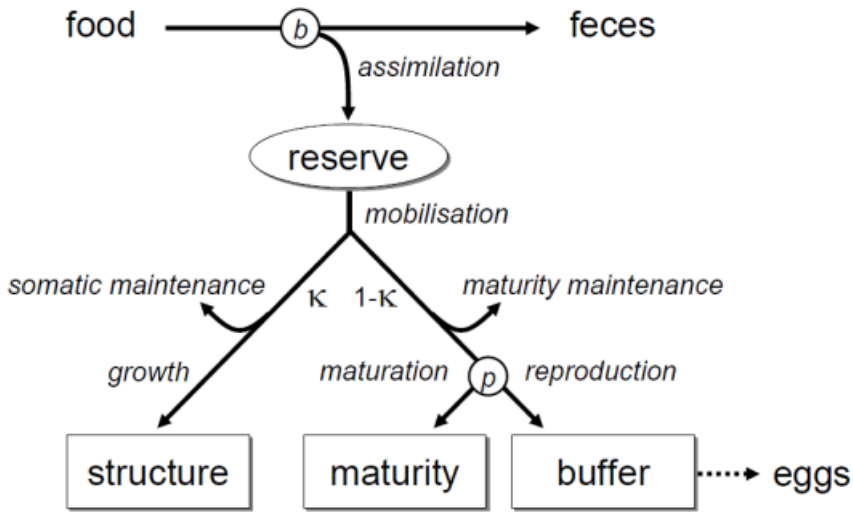


Figure 1.2: Schematic description of the DEB framework (the κ - rule). Boxes indicate the state variables involve in the model and arrows indicate energy/mass fluxes. The circles containing "b" and "p" denote maturity switches for birth and puberty, where feeding and allocation to reproduction begin respectively.

take rate is proportional to the environmental concentration, and the elimination rate is proportional to the internal concentration. This internal concentration translated to effects on endpoints using a toxicodynamics (TD) model. The TD model comprises a model that links internal concentrations to effects on one or more of parameters of the energy budget and the DEBkiss model to translate that to endpoints over time.

1.6 Extrapolating the individual-level effects to population

To address the different extrapolation issues considered in this thesis, I use matrix models and the Euler-Lotka equation. The potential use of matrix models to extrapolate individual-level effects to population is discussed by a number of authors (e.g. [31, 35–37]). Generally, matrix models are easy to construct and interpret [36]. They offer the advantage of simplicity, not only in the modeling process of underlying biological phenomena, but also in the sensitivity analyses and the simulation running [31].

A particular interesting aspect of matrix population models is their capability to estimate asymptotic population growth rates: a population-level endpoint that is relevant for ecotoxicological applications [38]. Thus, the practical application of matrix models is usually aimed at

estimating the asymptotic population growth rate of an animal at different levels of toxicant exposure. The information to be derived helps to compare the asymptotic population growth rate for toxicant stressed and unstressed populations [36] and/or derive dose-response relationship. Common to all matrix models is that information on individual-level vital rates (e.g. survival, growth and reproduction) are the main data inputs. In ecotoxicology, these individual-level vital rates are usually derived from laboratory ecotoxicological tests in which individual-level effects (e.g., the effects on individual life span, fertility, and body growth) of toxic chemicals are measured. Unfortunately, these tests focus on effects at what are considered to be the most sensitive stages of the life cycle [11]. However, effects on the most sensitive life stages do not necessarily drive population-level effects. This limits the use of population models to extrapolate individual-level effects to the population level. Forbes and Calow [38], studied the relationships between effects of toxicants on population growth rate (r) and the individual-level traits and they found an inconsistent relationship between the effects of a toxicant on the population growth rate (r) and on individual life-history traits, contributing to it. This lack of consistency and unpredictable relationships between individual-level effect and population growth rate (r) make the extrapolation uncertain. It is also impossible to obtain population end-

points, like r , from the analysis of single parameters of individual life history [33]. Thus, if individual-level adverse effects of toxicants measured in standard bioassays are to be used as the main data sources to predict ecological risks, the bioassays should focus on testing effects on those demographic parameters which influence population growth rate to the largest extent.

In this study, I demonstrate how matrix models can also be used as a tool in the design of short-term ecotoxicological tests. I apply an age-based matrix model to analyze the effects of cadmium and imidacloprid (independently and in mixture) on aphids (*Acyrtosiphon pisum* Harris). I use the matrix model to estimate the population multiplication factor (λ) of the animal at different exposure levels (treatments). I then use a demographic decomposition analysis to estimate the contribution of changes on age-specific survivorship and fecundity values to the global difference in the population growth rate.

Lack of ecological realism in standard ecotoxicological tests is the other important issue in application of matrix models for the extrapolation of individual-level effects to populations. Standard ecotoxicological tests are standardized and conducted under resource unlimited and constant environmental conditions. Thus, matrix models parameterized using data obtained from these standardized tests are

deterministic and can be used to estimate the deterministic population growth rates (λ_d) at different toxicant exposure. The deterministic population growth rates help to assess whether population are in exponential growth ($\lambda_d > 1$) or decline ($\lambda_d < 1$). However, assessing the population-level effects of toxicants in the field using λ_d as end-point might lead to erroneous conclusion. In the natural environment, key ecological factors can modify effects of toxicants at individual and population levels. Particularly, fluctuations in environmental conditions can have a direct effect on survival and fecundity of individuals [36] and can have consequence for the relevance of the λ_d . Thus, more ecologically relevant population model can be developed by including the effect of environmental variability in the deterministic matrix models. This can be done by formulating stochastic matrix models under the assumption that the variation in vital rates among matrices is due to fluctuating environmental conditions [39, 40].

Stochastic matrix models provide information on the stochastic population growth rate and the variance due to environmental fluctuation. These end-points can be also used to estimate and predict important population-level end-points; extinction probability and time to quasi extinction. However, there are theoretical and practical issues pertaining to the formulation of stochastic matrix models for short-living organisms used in standard toxicity tests, thus limiting their

application in ecotoxicology and ERA process. In Chapter 3 of this study, I present a methodology for including environmental stochasticity arising from seasonal environmental variability. I demonstrate the practical applicability of the method using a case study, in which I develop a stochastic matrix model for my model organism: *Folsomia candida*. I also demonstrate how stochastic and non-linear matrix models can improve the extrapolation of individual-level effects to population-level in the natural environment.

Instead of matrix models, I use the Euler-Lotka equation to extrapolate the DEB based individual-level mechanistic effect model to the population-level. Figure 1.3 shows the conceptual framework of the link among these different elements of the model. The Euler-Lotka assumes a strictly constant environment and that every individual in every generation follows the same life-history. The selection and use of the matrix models and Euler-Lotka equation is based on two important factors: the research questions I wanted to answer and availability of data for parameterizing the population models. For instance to parameterize the Euler-Lotka equation, we need survival and reproduction as continuous functions of time. These life history data can be easily obtained from the DEBkiss model. However, parameterizing the Euler-Lotka equation using data derived from standard toxicity data is impossible without additional assumptions. Thus, I use matrix

models to extrapolate individual-level effects, which are derived from standard toxicity tests, to population-level. In contrast to the Euler-Lotka equation, the integration of a matrix model with DEBkiss is not straightforward, hence it requires a translation of DEBkiss results to the 'vital rates' in the matrix [41]. On the other hand, It is impossible to include density dependence or environmental stochasticity in Euler-Lotka equation.

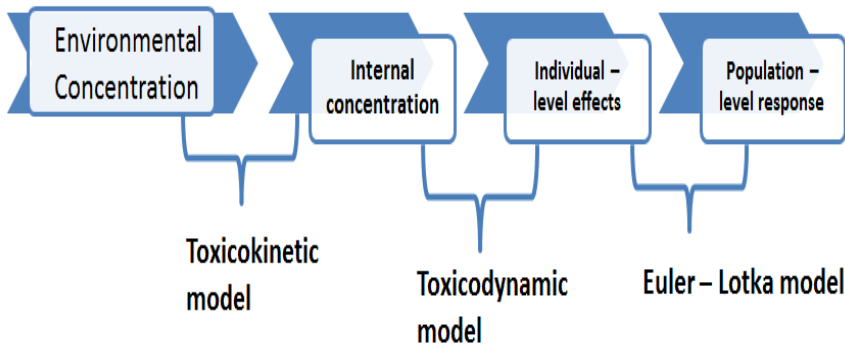


Figure 1.3: Conceptual framework of the energetic based mechanistic effect model coupled with Euler-Lotka model to extrapolate individual-level effects to population-level response

1.7 Study species and stressors

The modeling approaches I present in this thesis are generic and can be implemented to answer similar questions to those posed in this thesis. I demonstrate the applicability of these generic model frameworks

based on case studies by assessing the combined effects of selected stressors on my model organism.

1.7.1 *Folsomia candida* : model organism

The work is based on studies on a model species, *Folsomia candida*. *F. candida* is a common soil invertebrate and a standard bioassay species in both OECD and ISO standard protocols [42, 43]. The organism is well studied both in ecology and in ecotoxicology (described first by Willem (1902)), and belongs to the family Isotomidae [44]. Figure 1.4 shows a female adult *F. candida* with eggs and newly hatched juveniles on plaster of paris. As populations of *F. candida* consist exclusively of parthenogenetic females, they are easy to culture in the laboratory and populations of one genotype can be obtained. At 20 °C, OECD standard test temperature, they take between 21 and 24 days to reach the sixth, or adult, instar when they are sexually mature. About 30 to 50 eggs are laid in each batch, which take 7 to 10 days to hatch [45].

1.7.2 Stressors

In this thesis, I considered temperature as the main natural stressor. This choice is based on the fact that temperature is ecologically important and highly variable in the natural environment, which may cause stress responses on organisms. What it is more, the vital rates



Figure 1.4: A fully grown (≈ 2.5 mm long) adult *Folsomia candida* (Willem) with eggs and newly hatched juvenile on plaster of paris

of *F. candida* are temperature dependent. According to the review paper by Fountain and Hopkin [45], discussing the effect of temperature on life history of *F. candida* over its life time, the average number of eggs laid by a single female at 15, 21, and 27 °C is around 1100, 900, and only 100, respectively. The survival of *F. candida* is also temperature dependent. Snider and Butcher [46] studied the age specific survival and longevity of *F. candida* at three temperatures (15, 21 and 26 °C) and found that higher temperature reduces individual life span. Thus, temperature might interact with toxicants and influence the potential effects of chemicals.

In addition to temperature, I also considered crowding as additional stressor. The effect of crowding, which is caused by higher population density, on the vital rates of *F. candida* is reported by a number of authors. For example, Noel [44] found that crowding in *F. candida* populations results in reduced reproductive output and reduced somatic growth rate which, in turn, reduce population growth rate. Therefore, crowding might also influence the population-level effects of toxicants.

Cadmium was chosen as a model toxicant. Menta et al. [47] reported the effect of Cd on survival of embryos, survival of juveniles, and egg production at different concentration levels. Similarly, Crommentuijn et al. [48] reported the effect of Cd on survival of adults, development

rate of embryos, development rate of juveniles, and population increase at different concentrations.

1.8 Thesis Organization

Including this Introduction, the thesis is organized in seven main chapters and a Summary. In Chapter 2, I present a method that can help to determine the optimal approach for designing ecotoxicological tests considering population-level effects as endpoints. I present a demographic decomposition method, based on a typical Life Table Response Experiment (LTRE), allowing for selecting the most relevant life stages for individual-level toxicity tests. The method is illustrated using data on the toxic effects of cadmium (Cd) and imidacloprid on reproduction and survival of aphids (*Acyrtosiphon pisum* Harris). Chapters 3, 4 5 and 6 present the modeling approaches I used to answer the main questions posed in this study, relating to the combined effects of chemicals and natural environmental factors. In Chapter 3, I evaluate different methods for estimating the environmental stochasticity arising from seasonal environmental fluctuations and present a methodology for developing stochastic matrix population models. Although stochastic matrix models are widely used to predict effects of environmental fluctuations on the population dynamics, their application in estimating effects of environmental stochasticity to popu-

lations impacted by toxic chemicals is limited. This is mostly because the available methods for estimating stochasticity in populations rely on life-history data which are very difficult, if not impossible, to obtain for animals with a short life span, such as most animals used in standard bioassays. In that chapter, I present a modeling technique that allows estimating environmental stochasticity arising from seasonal environmental variability, with emphasis on organisms with short life span. The applicability of the model is illustrated by considering the effect of seasonal temperature fluctuations on the vital rates of *F. candida*.

Chapter 4 deals with the stochastic density-dependent matrix (SDDM) model I developed for extrapolating the effects of chemicals on individuals to populations in the natural environment. I present a generic matrix population model for assessing the ecological risks of chemicals to populations in the field by accounting for density-dependence and environmental stochasticity arising from seasonal environmental fluctuations. As an illustration, I present a case study that predicts effects of cadmium (Cd) on density-dependent populations of *F. candida* living in environments with temporal and/or spatial temperature variation.

In Chapter 5, I present the basis of the simplified DEBkiss model developed for our focus organism *F. candida* in the absence of tox-

icant exposure. The most important step in developing a species-specific DEBkiss model is its parameterization. Thus, in that chapter, I present how the various model parameters can be obtained by performing simple life-cycle experiments and observing standard end-points at different environmental conditions. I present model simulation results demonstrating the model's capability in predicting the influence of food limitation and temperature on life history of *F. candida*. The model is further linked with the Euler-Lotka equation to predict the effect of food availability and temperature on the population growth rate of *F. candida*. The same DEBkiss model was implemented in Chapter 6, where I integrate toxicokinetic (TK) and toxicodynamic (TD) models with the basic DEBkiss model and develop a mechanistic individual-level effect model. The model is applied to analyze the combined effects of temperature and cadmium on the life history of *F. candida*. The model is also used to understand the underlying mechanisms responsible for the observed interactions between the two stressors. This individual-level effect model was further linked with the Euler-Lotka equation to predict the combined effects of Cd and temperature on the intrinsic population growth rate (r) of *F. candida*.

Finally, in Chapter 7, I summarize my findings, emphasizing the predictability of the combined effects of chemicals and environmental fac-

tors, discuss its implications in the context of ERA, indicate potential areas of improvements and outline future research requirements. Finally, I conclude the overall findings of my work, discuss its limitation, and provide suggestions for future works.

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Chapter 2

Decomposition analysis of LTREs may facilitate the design of short-term ecotoxicological tests

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Abstract

This study compared two methods, based on re-analyzed data from a partly published Life Table Response Experiment (LTRE), to help determine the optimal approach for designing ecotoxicological assessments. The 36-day LTRE data recorded the toxic effects of cadmium (Cd) and imidacloprid, alone and in combination, on the reproduction and survivorship of aphids (*Acyrtosiphon pisum* Harris). We used this data to construct an age-classified matrix model (six age classes, each six days long) to estimate aphid population growth rate (λ) under each treatment. For each treatment, an elasticity analysis and a demographic decomposition analysis were performed, and results were compared. Despite different results expected from the two toxicants, the elasticity values were very similar. The elasticity of λ with respect to survival was highest in the first age class, and that with respect to fertility was highest in the second age class. The demographic decomposition analysis examined how changes in life-history traits contributed to differences in λ between control and treated populations ($\Delta\lambda$). This indicated that the most important contributors to $\Delta\lambda$ were the differences in survival (resulting from both demographic sensitivity and toxicity) in the 1st and the 2nd age classes of aphids and differences in fertility in the 3rd and the 4th age classes. Additionally, the toxicants acted differently. Cadmium reduced $\Delta\lambda$

by impairing fertility at 3rd age class and reducing survivorship from the 2nd to the 3rd age class. Imidacloprid mostly reduced survivorship at the first and second age classes. The elasticity and decomposition analyses showed different results, because these methods addressed different questions about the interaction of organism life history and sensitivity to toxicants. This study indicated that the LTRE may be useful for designing individual-level ecotoxicological experiments that account for both the effects of the toxicant and the demographic sensitivity of the organism.

Key words: Life Table Response Experiment, matrix population model, decomposition analysis, elasticity, *Acyrtosiphon pisum*, ecotoxicological assays

2.1 Introduction

The ecological risks of toxic chemicals are typically assessed on the basis of individual survival, reproduction, or growth. These data might be more effectively used to predict the effects of toxic chemicals at higher levels of ecological organization. In fact, the protection aims of various Ecological Risk Assessment (ERA) schemes commonly focus on populations, communities, or entire ecosystems [1]. Thus, the impact of toxicants on an individual life history is typically extended to the population level with mathematical models designed to calculate the main demographic indices and other population statistics. In particular, the population growth rate (λ), which takes into account survival, development time, and reproduction, can provide valuable information that cannot be obtained from the analysis of single parameters of individual life history. Previously, Forbes and Calow [2,3] and Barnthouse et al. [4] discussed the suitability of using population growth rate as a measure of population-level toxicant effects. Among different mathematical modeling approaches, one of the most robust techniques is matrix population modeling. In that approach, individual level data is used as an input to calculate characteristic endpoints for the population [5].

Traditional ecotoxicological testing often determines a dose-effect relationship for a given chemical on a certain endpoint (like survival or

reproduction) at a fixed time point. Although these tests represent a standard in ecotoxicology, the data generated are only applicable to effects on the measured endpoint at one specific time point. These short-term assays have several drawbacks for environmental risk assessments, which have been well scrutinized by various authors. For example, Laskowski [6] showed that these short-term assays neglected the cumulative nature of some chemicals and the potential accumulation of toxic effects. The author also pointed out that short-term assays neglect effects on outcomes other than mortality and fecundity and account for only a small part of the life history of an organism. Therefore, they do not provide any information on population dynamics under toxic stress. On the other hand, conducting a full life cycle study in a Life Table Response Experiment (LTRE) is lengthy, impractical, and expensive. Thus, there is a need for ecotoxicological tests that can more efficiently capture toxic chemical effects on the population level, based on individual-level responses. Demographic perturbation analysis can be a powerful tool for developing such tests. It explores how population statistics respond to changes in the vital rates using two logically distinct ways [7]. Prospective analyses (e.g. elasticity) explore the functional relationship between population growth rate and the vital rates, while retrospective analyses (e.g. demographic decomposition of LTREs) enables the variation

in λ to be expressed as a function of variation in vital rates [7, 8]. The extent to which λ responds to toxicant exposure depends on the severity of the toxicant effect on individual life-history traits and the sensitivity of λ to changes in the individual traits that contribute to it [9]. Hence, based on the information obtained from a demographic decomposition of LTRE, we can pinpoint the most ecotoxicologically important life stages of an organism; i.e. the life stages that capture most of the severity of toxicant's effect on λ . This technique can be considered an empirical extension of a sensitivity analysis; it allows one to analyze data from chronic toxicity tests to assess how the toxicant impact on each vital rate contributes to the overall impact on population growth [10]. Due to the broad use of some of the terms used in this area, we would like to define the following terms: elasticity in this work refers to the (mathematical) demographic sensitivity of λ to changes in vital rates; and contribution represents the combined effects of demographic sensitivity and the response of vital rates to toxicants on λ . These strict definitions of terms are intended to avoid subsequent confusion. The main objective of this study was to compare results from retrospective (demographic decomposition of LTRE) and prospective (elasticity analysis) approaches to highlight potential differences in outputs and the potential consequences of applying each method in ecotoxicology testing and risk assessment. The

demographic decomposition of LTRE data also allowed a comparison of the contributions of each vital rate to the actual changes in the population growth rate in treated and control groups ($\Delta\lambda$). The results were used to identify the most important aphid life stages that, when investigated at the individual level, can capture population-level impacts of toxicants. Because the data originated from the full factorial experiment, we were able to perform a comparison between single-chemical treatments and combined-chemical treatments.

2.2 Materials and methods

2.2.1 Experimental design

The data for this study originate from an experiment performed by Laskowski [6]. Briefly, the aphids (*Acyrtosiphon pisum* Harris) were bred on potted broad bean plants, planted in 250 g dry weight garden soil. The full factorial design was used, with two Cd treatments (100 and 200 mg per kg dry weight), two imidacloprid treatments (4 and 40 g.a.i per ha.) and the four possible combined treatments assigned among the pots at random before planting the beans. Cd contamination was achieved by watering the soil with 100 ml of an appropriate CdCl_2 solution (anhydrous, ACS grade in distilled water) before the beans were planted. Controls were treated with an equal volume of distilled water. After three weeks, imidacloprid contamination was

achieved by adding imidacloprid (BAY NTN 33893, 17.4% active ingredient) in 50 ml distilled water directly to the soil surface of each pot. The doses were approximately equivalent to $0.01\times$ and $0.1\times$ of the recommended field dose. This resulted in nominal concentrations of ca. 14.4 and 144 ng a. i. per g of dry soil, respectively. Controls and other treatments received 50 ml distilled water. Four days later, only three plants per pot were kept, and aphids were introduced to the plants. The adult reproducing aphids were placed individually into clip cages, one per pot; after 24 h, the adults and all neonates except one per cage were removed. Every clip cage was monitored daily until the death of the aphid; and all newborn neonates were counted and removed. When the leaf with a clip cage started to wilt, the cage and aphid were moved to a new leaf; when the whole plant wilted, they were moved to another plant in the same pot. Throughout the experiment, the pots with plants remained in plastic trays filled with tap water. The experiment ended after 36 days, when all the aphids died.

2.2.2 The matrix model

An age-structured matrix projection model, known as the Leslie matrix model, was used to investigate the population-level effects of cadmium and imidacloprid applied alone and in mixtures. For this, the

individual daily life-history data of aphids were recalculated and organized in cohort life tables. The aphids lived for a maximum of 36 days; hence, we used life tables with 6 age classes, each 6 days long (i.e., 1 – 6, 7 – 12, ..., 31 – 36 days). The life tables were used to parameterize age-classified projection matrices, and the entries were rounded to the nearest second decimal digit for survival rates.

The basic structure of the Leslie matrix is given in equation 2.1.

$$\begin{bmatrix} F_1 & F_2 & F_3 & F_4 & F_5 & F_6 \\ P_1 & 0 & 0 & 0 & 0 & 0 \\ 0 & P_2 & 0 & 0 & 0 & 0 \\ 0 & 0 & P_3 & 0 & 0 & 0 \\ 0 & 0 & 0 & P_4 & 0 & 0 \\ 0 & 0 & 0 & 0 & P_5 & 0 \end{bmatrix} \times \begin{bmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \\ N_4(t) \\ N_5(t) \\ N_6(t) \end{bmatrix} = \begin{bmatrix} N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \\ N_4(t+1) \\ N_5(t+1) \\ N_6(t+1) \end{bmatrix} \quad (2.1)$$

F_i represents the fecundity of age classes 1 – 6; P_i represents the probability of survival from age class i to $i+1$; and $N_i(t)$ and $N_i(t+1)$ represent the numbers of individuals in age class i at time t and $t+1$, respectively.

2.2.3 Elasticity analysis

Elasticity analysis explores the functional dependence of λ on the individual vital rates. It has traditionally been used to determine

to what extent particular vital rates are important for population dynamics (measured by λ) of a species characterized by a specific life history. Thus, the elasticity of a matrix element, ε_{ij} , is the product of the sensitivity of a matrix element (S_{ij}) times the matrix element itself (a_{ij}), divided by λ . In essence, elasticities are proportional sensitivities, dimensionless, and normalized to between 0 and 1 to facilitate comparison among vital rates with different scales.

Mathematically, the sensitivity $\frac{\partial \lambda}{\partial a_{ij}}$ can be calculated as follows [5]:

$$S_{ij} = \frac{\partial \lambda}{\partial a_{ij}} = \frac{\bar{v}_i w_j}{\langle v_i, w_j \rangle} \quad (2.2)$$

where w and v are corresponding right and left eigenvectors of the largest eigenvalue, λ . Accordingly, elasticity of a matrix element can be calculated as follows [5],

$$\varepsilon_{ij} = \frac{\partial \log(\lambda)}{\partial \log(a_{ij})} \quad (2.3a)$$

$$\varepsilon_{ij} = \frac{a_{ij}}{\lambda} \times \left(\frac{\partial \lambda}{\partial a_{ij}} \right) \quad (2.3b)$$

Equations 2.3 are used here to calculate the elasticities for an untreated population. In addition, elasticities were calculated for each treated population (expressed in percentage):

$$\varepsilon_{ij}^T (\%) = \frac{a_{ij}^T}{\lambda^T} \times \left(\frac{\partial \lambda}{\partial a_{ij}^T} \right) \times 100 \quad (2.4)$$

The superscript T represents each treatment level. This elasticity analysis captured the toxic effects, and the output could be compared to results from the demographic decomposition of the LTRE.

2.2.4 Demographic decomposition of LTRE

Demographic decomposition methods express observed variations in λ as a function of observed (co)variations in the vital rates. The differences in population growth rates ($\Delta\lambda$) between the treated and control groups were analyzed according to the contributions of age-specific fecundities and survival probabilities. These contributions were identified based on the decomposition analysis technique proposed by [11], which was based on the Taylor's series expansion technique. In this formulation, $\lambda^{(T)}$ and $\lambda^{(C)}$ denote the values of λ for treated and control groups, respectively. The superscript T and C indicate the treatment (T) and control (C) groups. Then, the 1st order approximation of λ for toxicant-treated groups is given by taking $\lambda^{(C)}$ as the base of expansion, as follows:

$$\begin{aligned} \Delta\lambda &= \lambda^{(T)} - \lambda^{(C)} \\ &\approx \sum \left(a_{ij}^{(T)} - a_{ij}^{(C)} \right) \times \left(\frac{\partial \lambda}{\partial a_{ij}} \right) \Big|_{\frac{1}{2} \times (A^{(T)} + A^{(C)})} \end{aligned} \quad (2.5)$$

In the summation, the difference between treated and control values in each Leslie matrix element, a_{ij} (age-specific survivorship or fecundity values) was evaluated for its contribution to the global difference in the population growth rate, $\Delta\lambda$. In this way, it was possible to identify the life-history traits that had the greatest influence on λ for each treatment and compare the relative contributions of each trait to the $\Delta\lambda$ between the treated and control groups. As mentioned above and confirmed by Caswell [11] and Levin et al. [9], Equation 2.5 is an approximation; its accuracy should be verified prior to implementation to determine whether a higher order approximation should be used. Therefore, we conducted a preliminary analysis aimed at testing the accuracy of the equation to be applied to our LTRE by comparing the equivalency of the left and right hand sides (LHS and RHS) of equation 2.5.

The key to breaking-down the differences in population growth rates between the treated and control groups was to evaluate the terms inside the summation on the RHS of Equation 2.5. The RHS of Equation 2.5 contains two components. First, we calculated the difference between the matrix coefficients for the control and treated groups, $(a_{ij}^{(T)} - a_{ij}^{(C)})$; then, we evaluated the sensitivity of each matrix coefficient with respect to the λ value at the midpoint between the control and treated groups, $\frac{\partial\lambda}{\partial a_{ij}}|_{\frac{1}{2} \times (A^{(T)} + A^{(C)})}$. The former is a

straightforward subtraction. The latter calculation is equivalent to Equation 2.2, where the sensitivity is calculated at the midpoint between the control and treated groups. Thus, we needed to evaluate the λ value at the midpoint between the control and each treatment; then, we could evaluate the corresponding right and left eigenvectors. All calculations were performed with a program code developed in MATLAB. The built-in functions for matrix algebra were utilized to evaluate the demographic statistics (λ , w , and v), the sensitivities, and the contributions to treatment effects on λ .

2.3 Results

2.3.1 Parameter estimation

The individual life-history data were used to evaluate parameters in Equation 2.1. Once the values of F_i and P_i were determined, we developed Leslie matrices for each treatment. In the individual life history data, large differences were found between individual lifetimes and fecundity. Consequently, there was also a large variation in calculated population growth rates. Therefore, a bootstrapping procedure was applied, where the data were reassembled 5000 times. Table 2.1 summarizes the vital rates analysis results and the estimated population growth rates.

Table 2.1: Probability of survival, P_i , fecundity, F_i , and mean population growth rate, λ , (day^{-1}) for different treatments (Treatment levels are represented as the Cd concentration in mg kg^{-1} /imidacloprid dose in g a.i. ha^{-1})

Age class	Treatment Levels								
	0/0	100/0	200/0	0/4	0/40	100/4	200/4	100/40	200/40
P_i									
1	0.89	0.90	0.68	0.50	0.46	0.74	0.88	0.33	0.71
2	0.94	0.83	0.54	0.83	0.67	0.86	0.71	0.75	0.80
3	0.77	0.53	0.29	0.60	0.75	0.42	0.30	0.33	0.38
4	0.54	0.25	0.00	0.33	0.33	0.00	0.00	0.00	0.00
5	0.31	0.00	-	0.00	0.00	0.00	-	-	-
6	0.00	-	-	-	-	-	-	-	-
F_i									
1	1.5	2.3	0.8	3.7	0.0	2.4	2.3	0.0	0.4
2	50.2	44.4	29.6	39.7	12.8	41.9	35.0	11.5	19.6
3	27.0	19.8	8.5	11.7	18.5	16.9	8.6	11.0	15.5
4	1.6	1.9	0.0	0.5	0.0	0.3	0.0	0.0	0.7
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
λ , day^{-1} , values in brackets represent standard deviations									
λ	1.282	1.274	0.829	1.125	0.469	1.172	1.148	0.701	0.373
STD	(0.117)	(0.172)	(0.196)	(0.237)	(0.198)	(0.208)	(0.195)	(0.164)	(0.222)

2.3.2 Elasticity analysis

The elasticity analysis was first performed with a 'traditional' method; that is, the elasticities were calculated for an untreated population (Figure 2.1 and 2.2). The elasticity of λ with respect to survival was highest in the first age class, and that with respect to fertility was

highest in the second age class. The elasticity with respect to fertility was also high in the first age class, despite the low reproduction rate compared to the 2nd and 3rd age classes (Table 2.1). The fertility elasticities were also calculated for each treatment separately (Figure 2.1). In the second age class, λ was highly sensitive to changes in fertility with all treatments. In the first age class, λ was sensitive to changes in fertility under all treatments, except the three with the high imidacloprid dose. This was a direct result of the fact that aphids did not reproduce at all in the first age class at imidacloprid = 40 g a.i. ha⁻¹ (Table 2.1). For all other age classes, the λ sensitivity to changes in fertility was very low or zero with all treatments. A different pattern emerged for treatment-specific survival elasticities (Figure 2.2). In the first age class, λ was highly sensitive to changes in survival with all treatments. For the second age class, the λ sensitivity was very low; for all other age classes, the effects were negligible.

2.3.3 Accuracy analysis of numerical approximations

As mentioned above, prior to the detailed mathematical formulation of the LTRE analysis, we evaluated the accuracy of the first order numerical approximation shown in Equation 2.4. The accuracy of the first order approximation was evaluated by comparing the left and right hand sides (LHS and RHS) of Equation 2.4. The accuracy was

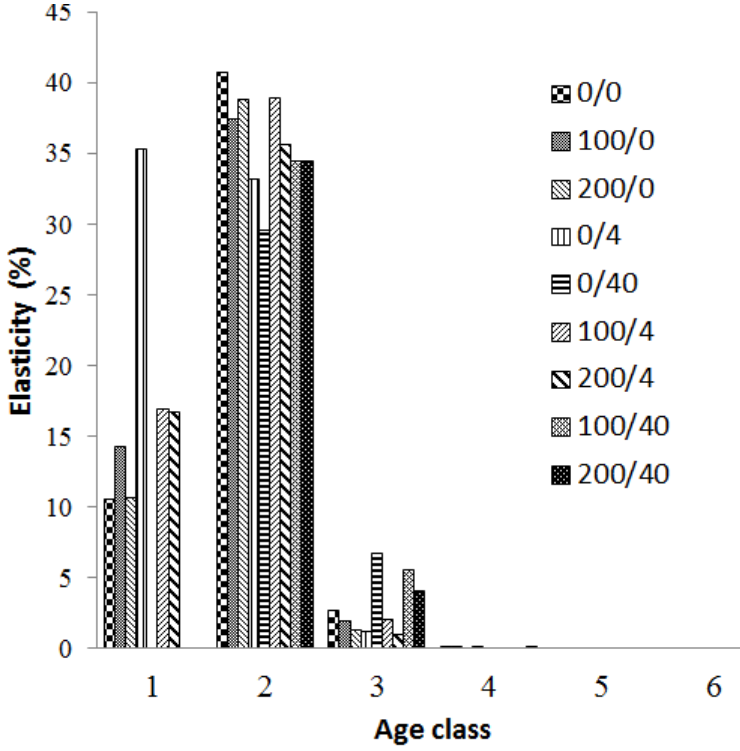


Figure 2.1: Control and treatment-specific lambda elasticities to changes in reproduction rates. The treatments are represented as Cd concentrations in mg kg^{-1} and imidacloprid dose in g a.i. ha^{-1} ; 0/0 stands for control.

reflected in the fit of the calculated values to the actual data. The results showed that the largest difference between the approximated values and the actual data was 0.189. Except for the treatment level of 200/40, the calculated values did not differ from the actual data by more than 3%. This value is estimated to be 10.9% for the 200/40

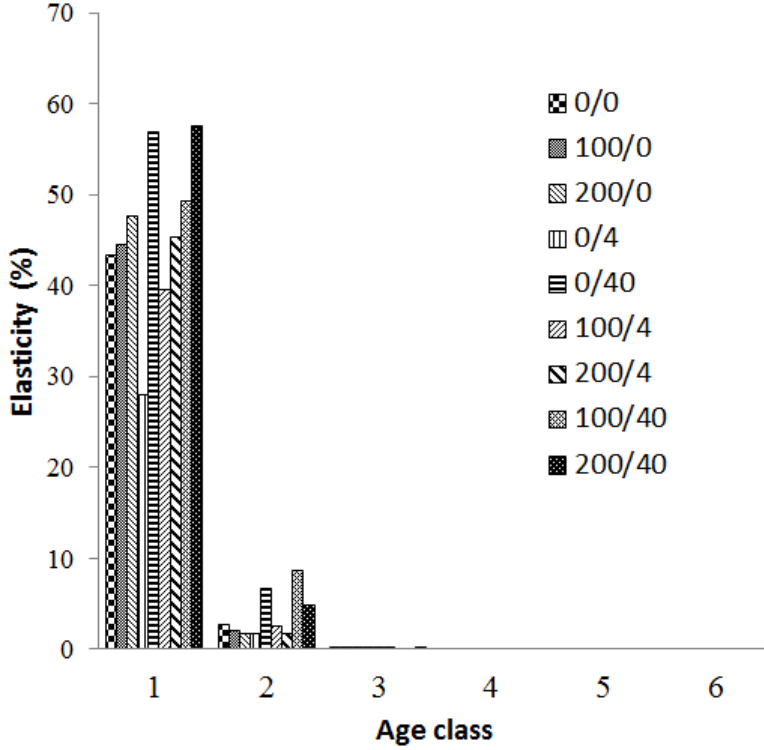


Figure 2.2: Control and treatment-specific lambda elasticities to changes in survival probabilities. The treatments are represented as Cd concentrations in mg kg^{-1} and imidacloprid dose in g a.i. ha^{-1} ; 0/0 stands for control.

treatment level. Therefore we assumed the first order expansion to be good approximation of the differences in population growth rates between the treated and control groups. Based on this first order approximation, we calculated the contribution of each matrix element to the global difference in population growth rate, $\Delta\lambda$, and the dif-

ference between each treatment and the control group was evaluated. Thus, it was possible to identify the life-history traits that had the greatest influence on λ , considering specific treatment effects. Then, we could compare the relative contributions of each vital rate to the difference in λ between the treated and control groups. Accordingly, the LTRE decomposition results provided the basis for understanding which life-history traits were most suitable for focused, short-term toxicity tests. The results pinpointed the stage-wise contributions of toxic effects on fertility and survival.

2.3.4 Effect on fertility

All cadmium treatments had a negative effect on aphid fertility. At the low Cd concentration (100 mg/kg), the effect on fertility was limited to the 3rd and 4th age classes. At the higher concentration (200 mg/kg), the effects went beyond the 3rd and 4th age classes, and reduced the fertility from the 2nd to the 5th age classes. However, the effects in the 2nd and 5th age classes were smaller than those in the 3rd and 4th age classes. Generally, imidacloprid also had a negative effect on aphid fertility. However, at the low treatment rate (4 ng a.i. ha⁻¹), the effect was quite surprising. At this low level, imidacloprid affected the fertility of aphids positively in the 2nd age class, and negatively in the 3rd, 4th, and 5th age classes. The negative effect was strong in

the 3rd and 4th age classes. At the higher imidacloprid concentration (40 ng a.i. ha⁻¹), the fertility of aphids was reduced further, and the effect was extended from the 2nd to the 5th age class. The effect was most pronounced in the 3rd and 4th age classes, particularly in the 3rd age class. In the combination of Cd and imidacloprid, the effect on fertility was most pronounced in the 3rd and 4th age classes. The effect increased abruptly at the high concentration of imidacloprid. The analysis of the proportional contributions of these effects to the population growth rate (λ) clearly indicated that both contaminants had the largest effects on the reproduction rate in the 3rd age class, which significantly contributed to λ (Figure 2.3). This was due to a combination of the strong effects of the contaminants on aphid fertility at this age and the heightened sensitivity of the population growth rate (λ) to changes in aphid fertility in the 3rd age class (cf. elasticity analysis above).

2.3.5 Effect on survival probability

In general, both Cd and imidacloprid had negative impacts on the survival of aphids. At the low Cd concentration (100 mg/kg), mortality decreased in the 1st age class compared to controls. Unlike the effects on fertility, the negative effects of these contaminants on survival were pronounced for all age classes. However, because the age

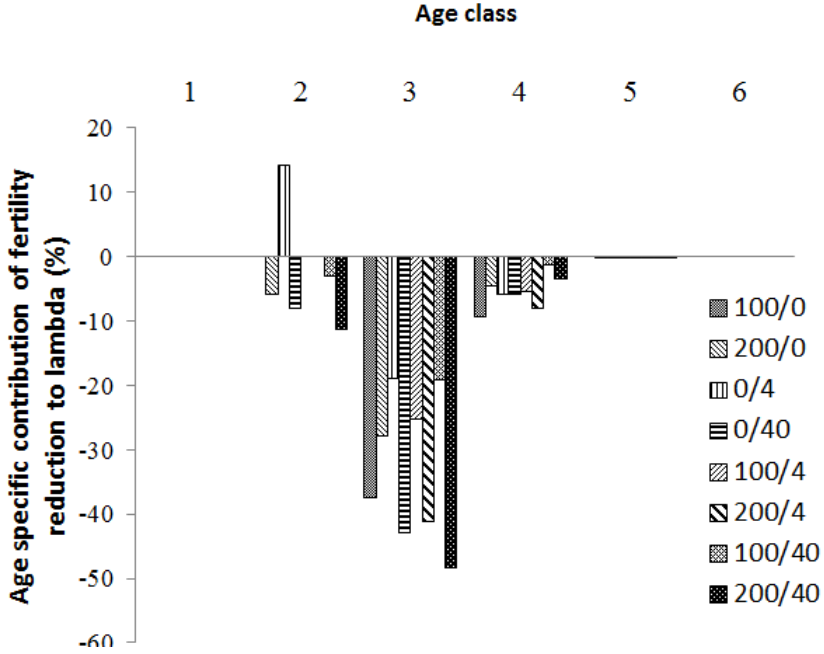


Figure 2.3: Treatment-specific contributions of reductions in fertility to the change in λ . The treatments are represented as Cd concentrations in mg kg^{-1} and imidacloprid dose in g a.i. ha^{-1}

classes had different demographic sensitivities, they contributed quite differently to the reduction of λ (Figure 2.4). Our results showed that the first two age classes made the most important contributions to the reduction of λ in terms of the overall decrease of λ in the treated population.

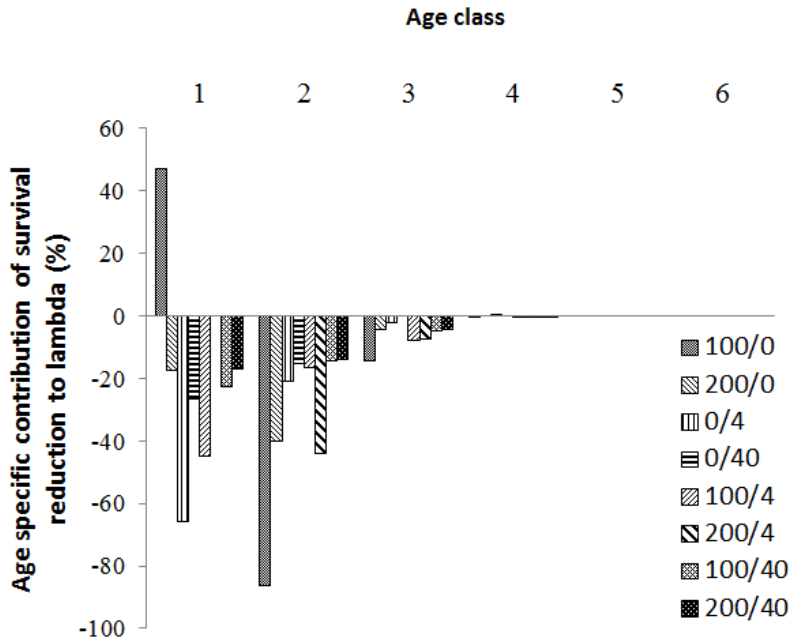


Figure 2.4: Treatment-specific contributions of survival reduction to the change in lambda. The treatments are represented as Cd concentrations in mg kg^{-1} and imidacloprid dose in g a.i. ha^{-1} ; 0/0 stands for control.

2.4 Discussion

The toxic effects of insecticides on aphid population growth rates are due to their negative effects on two individual life-cycle traits: survival probability and fertility. This study showed that these effects differed among aphid age classes; furthermore, the population growth rate, λ , was not equally sensitive to changes in the different vital

rates. Several studies have shown that susceptibility to toxic effects varies among different aphid life stages [12, 13]. Therefore, we were not surprised to find differences in λ sensitivities to vital rates that corresponded to specific life stages. Furthermore, one could expect that cadmium and imidacloprid might act in a different manner. As noted by Laskowski [6], these two toxicants represent two completely different classes of chemicals. Imidacloprid kills rapidly and degrades; in contrast, cadmium slowly accumulates, does not degrade, and exhibits its effects in later age stages. Consistent with this, we found that the imidacloprid-induced reduction in survival made the highest contribution to the change in λ in the first age class, while cadmium made the highest contribution to the change in λ in the second age class.

Surprising effects were found in mixtures of the two toxicants. At high Cd levels (200 mg/kg), λ was higher in the presence than in the absence of imidacloprid (200/0 < 200/4); and at high imidacloprid levels λ was higher in the presence than in the absence of cadmium (0/40 < 200/40). This indicated that an interesting dose-dependent interaction must have occurred between the toxicants, which appeared to be antagonistic. Unfortunately, the study did not provide any further information that might point to possible physiological mechanisms behind these effects. However, it is important to stress that, for

all four mixtures, the contributions of survival reduction to λ never exceeded those of single toxicants at the same concentration. Unlike the contributions of survival reduction, contributions of fertility reduction to λ did not differ substantially between treatments. Whether the toxicant was applied alone or in a mixture, effects on the third age class contributed most to λ reduction. On the other hand, traditional elasticity analysis suggested that λ was most sensitive to reductions in vital rates in the first two age classes. This strongly differed from decomposition analysis results. Similar differences were reported by other authors [14,15]. However, these two methods addressed different questions about the sensitivity of organisms to toxicants. The different results were a direct consequence of the different mathematical formulations for elasticity and decomposition. As indicated in Equation 2.3, the calculation of traditional elasticities depended solely on the projection matrix elements, a_{ij} . The effect of the toxicant on the vital rates was not included, and thus, it did not influence the elasticity values. The toxic effects could be captured by performing an elasticity analysis for each treatment; thus, the toxic effect could be included by comparing the demographic sensitivities among the treated and untreated populations. Nevertheless, this elasticity analysis would not pinpoint the most toxicant-sensitive age classes. This fact was supported by our treatment-specific elasticity results for both

survival and reproduction. As shown in Figure 2.1 and 2.2, despite the different results expected from the two toxicants, the elasticity values were very similar. The highest elasticity values were found for reproduction in the 2nd age class and survival from the 1st to the 2nd age class. This was due to the fact that elasticities depend on the functional dependence of λ on all a_{ij} entries in addition to the local, functional relationships among the a_{ij} [7]. The low elasticity of environmentally sensitive life-cycle traits was previously discussed by Forbes et al. [16]. Based on published studies relevant to ecotoxicological testing, they showed that elasticity was negatively related to the life-cycle trait sensitivity to toxicants in all studies. This implied that natural selection favors reduced elasticities in life-cycle traits that are sensitive to environmental variations. It is difficult to disagree with that statement in general terms; however, our results indicated that eventual toxicant effects on λ , which are the outcome of the effects on particular vital rates combined with their elasticities, could differ substantially between fecundity and survival rates and among chemicals. For example, although the reductions in fertility indeed contributed most to $\Delta\lambda$ in age classes of relatively low elasticity, this was not the case for survival rates. The effects of the chemicals on survival were similarly substantial in all age classes; therefore, the eventual toxic effects of all treatments showed that changes in survival made

the largest contributions to $\Delta\lambda$ in the first age class, which had the highest elasticity.

Elasticity analysis has been proven useful in ecotoxicological research. By applying a simple two-stage population model, Hansen et al. [17] manipulated life-history parameters measured in laboratory-reared animals to simulate potential effects of competition and predation on vital rates in order to explore how such factors might influence the sensitivity of population growth rate to toxicant-caused changes in individual life-history traits. Hansen et al. [17] showed that effectively predicting the population-level consequences of toxicant effects estimated on individual level can be improved by exploring the elasticity pattern of λ for the population over a range of ecological conditions. This work provides a good example of linking the effects of toxicants on individual organism performance to effects at the population level of organization. Nevertheless, one should be careful when interpreting elasticity analysis results or comparing them to outputs of different methods.

For the case of comparing the results of the decomposition and elasticity analyses, it is important to keep in mind that the underlying logic is different for the two approaches. Decomposition expresses the observed variation in $\Delta\lambda$ as a function of the observed (co)variation in the vital rates; thus, its results are specific to the observed pat-

tern of variation. Elasticity predicts the changes in λ that would result from any specified change in the vital rates; thus, its results are independent of variability patterns in the vital rates ([7]). The choice of method should depend on what it is being used to investigate. Elasticities have a place in life-history and conservation studies (see [18]); they are considered a good choice for identifying management targets or planning experiments ([10]). However, in ecotoxicology, certain precautions must be taken. Salice and Miller [14] stated that “decomposition analysis indicated that the vital rates that were altered when populations were exposed to Cd were not well predicted by elasticities”. Raimondo and McKenney [15] concluded that “although elasticity analysis identifies the life stages that may be key targets for conservation efforts ... the magnitude of change in population parameters is an equally important factor to consider during population-level risk assessment of toxicant exposure”. Hansen et al. [19] combined the two methods, rather than comparing them; the authors used elasticities to select sensitive stages, and decomposition analysis to determine whether the contributions of those stages were significant in reducing the population growth rate.

However, in the present study, the elasticity analysis missed the importance of the third age class, which turned out to contribute most to the λ reduction. Therefore, choosing the most important stage

based solely on elasticity analysis would be misleading; for example, it could lead to the conclusion that running exposure tests for 2 weeks would be sufficient to estimate toxic effects. For the sake of designing short-term ecotoxicological tests, the LTRE decomposition analysis appeared to be an efficient method on its own. Unfortunately, it requires substantially more data than the elasticity analysis. In addition to the life history of a species, it requires at least some preliminary data on the responsiveness of the life stages/age classes. Nevertheless, in terms of ecological relevance and acceptable cost, this avenue may be the most meaningful, particularly for long lived, iteroparous organisms.

A decomposition analysis requires a LTRE; but ecotoxicological studies that incorporate LTREs are relatively scarce. Caswell [20] noted that "*the full power of the LTRE approach ... has not yet been applied to ecotoxicology, but there is no reason it cannot be*". Unfortunately, in the past 15 years, there has not been a break-through in the use of LTREs in ecotoxicology; altogether, less than 50 papers have been published on the subject. Stark et al. [21] emphasized that the main disadvantage of this approach is that life table development is time consuming and expensive. However, it might be possible to run just one full LTRE per species for each major class of chemicals with the same mode of action; then, based on that foundation, we could design

tests specific for one species to test each class of chemicals. We argue that the benefits of this approach may supersede its disadvantages.

2.5 Conclusion

This study showed that a population-level interpretation of individual-level experimental results required information on two fronts; first, on how the toxicant affected the life stage under study, and second, on how sensitive λ was to changes in particular life stages. Our analysis combined experimental data on toxicant age-specific effects on vital rates with a sensitivity analysis of the aphid life history. A first order, fixed design-based LTRE [5] was used to identify age-specific impacts of the two toxicants. The analysis identified which vital rates made the largest contributions to the reduction of the population growth rate, $\Delta\lambda$, under specific treatments.

The approach presented in this paper can be used to identify and select the most relevant life stages for individual-level toxicity tests. Ecological risk assessment tests should be designed to determine how toxicity in particular life stages contributes to population dynamics. This can be determined by assessing the sensitivity of λ to changes in particular vital rates combined with the degree of change in a vital rate due to toxic impact. Our study showed that these contributions were different for different chemicals; this suggested that the design

of bioassays should be chemical-specific. For example, a test used for highly toxic, rapidly acting chemicals, like most organic pesticides (exemplified by imidacloprid in this study), should be designed differently from a test used for chemicals with low toxicity that accumulate in organisms, like metals (exemplified by Cd herein).

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Chapter 3

Incorporating environmental stochasticity into matrix population models: special emphasis on species with short lifespan

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Abstract

Stochastic matrix models are useful for translating the effects of environmental variability on individuals to populations and are frequently used by wildlife managers and conservation biologists. There are a variety of mathematical and statistical tools available to estimate environment-dependent life history parameters and estimate stochasticity arising from this variability. Among these tools, the analytical method proposed by Tuljapurkar [1] provides a mathematically tractable and robust method. This method requires three important pieces of information: environment-dependent deterministic projection matrices, an environmental state transition matrix, and the probability of occurrence of each of the environmental states. For many small animals, particularly for species with short lifespan, collecting exact environment-dependent demographic data in the field is not feasible from a technical and/or economical point of view. In this paper, we develop a methodology which combines easily available and/or measurable laboratory-based demographic data with actual field-measured climate records in order to overcome this limitation. As an illustration, we provide a case study for estimating effects of environmental stochasticity arising from temporal and/or spatial temperature variation on the springtail *Folsomia candida* (Willem) population, although our approach is by no means limited to this

species or environmental factor.

Key words Stochastic matrix models, Markov Chain probability matrix, Tuljapurkar's analytical method, Taylor's series approximation, climate change, risk assessment

3.1 Introduction

Matrix population models have become one of the most important tools to predict the dynamics of age or stage classified populations [2]. The practical applicability of matrix models ranges from assessing population health and trends in conservation biology through population viability analyses (PVA) and predicting the adverse effect of chemical stressors on population level [2–8]. Common to all matrix population models is that information on individual-level vital rates is the main data input. These individual-level vital rates are usually estimated by collecting data on survival, reproduction and/or growth for a specific period of time under standardized experimental setups or from field observations. Yet it is impossible to collect data for all possible environmental conditions in which different populations of a particular species actually live. Thus, all matrix population models based on such data are only snapshots of the whole system and this can hamper the usefulness of the models. One common approach to overcome this shortcoming is to include environmental stochasticity and formulate stochastic matrix population models under the assumption that the variation in vital rates among matrices is due to fluctuating environmental conditions [2, 5, 6, 9].

In general, environmental stochasticity can be incorporated into matrix models by either selecting deterministic matrices at random at

each time step of a simulation, or by selecting the vital rates at random from some statistical distribution [10]. Based on these two generalized approaches, three methods are currently used to formulate and analyze stochastic matrix models. Fieberg and Ellner [9] technically categorized these three methods as “random transition matrix” (RTM); “parametric matrix method” (PMM) and “small fluctuations approximation” (SFA); an analytical method which was proposed by Tuljapurkar [1] (see [9] for detailed discussion of the methods).

The development and implementation of all of these methods initially focused on ecology, resource management and conservation of endangered species. In recent years, there have been attempts to formulate stochastic matrix models for laboratory-reared organisms; particularly for estimating the effect of environmental stochasticity to populations impacted by toxic chemicals (e.g. [7]). The usual practice is to use the RTM, in which the environment is linked to vital rates by selecting laboratory-based demographic data at random from some statistical distribution. Hence, the method allows the vital rates to vary independently. In the natural environment, it is expected that there will be co-variation among the vital rates. In addition, assumptions for statistical distributions of the vital rates are based on demographic data collected from laboratory studies conducted under constant conditions and thus represent mostly individual phenotypic

variance rather than variability resulting from environmental conditions. Thus, the RTM method is the most uncertain technique for including environmental stochasticity in models based on laboratory-generated demographic data.

Considering PMM and Tuljapurkar's analytical method, we do not need to assume statistical distributions and it is also possible to include co-variation among the vital rates. Thus, these methods are preferable to estimate environmental stochasticity and formulate stochastic matrix models using laboratory-based demographic data. However, direct application of the methods is difficult and therefore they are typically used only for long-lived animals. Both methods require the derivation of environment-dependent matrices together with their frequency distributions, which are usually calculated based on year-to-year changes in vital rates. However, for species with a short lifespan, collecting environment-dependent demographic data from the field is often not feasible from a technical and/or economical point of view. Thus, the implementation of these methods for estimating environmental stochasticity for short-lived organisms needs different approaches, which take these issues into consideration.

This study aimed at designing a technique that would allow incorporating environmental stochasticity into matrix models for short-lived organisms. Considering the significant effect of temperature

on life history of short-lived organisms, we considered environmental stochasticity arising from temporal and/or spatial temperature fluctuations. Although the method is applicable to both PMM and Tuljapurkar’s analytical method, our work focuses on the Tuljapurkar’s analytical method, because it considers not only variability arising from inter-temporal (i.e. temporal variation and co-variation of vital rates) but also the effect of environmental autocorrelation on the population dynamics [9].

Our goal is threefold. First, we briefly discuss the mathematical background of Tuljapurkar’s analytical method. The method is the mathematical formulation for estimating the stochastic population growth rate and the environmental variance. Second, we discuss the step-by-step procedures for the estimation of parameters and variables, required for constructing and solving the analytical equation and highlight data requirements. Basically, the analytical method proposed by Tuljapurkar requires three important pieces of information: environment-dependent deterministic projection matrices, an environmental state transition matrix, and the probability of occurrence of each of the environmental states. The novelty of our work lies in the technique we used for deriving and evaluating these pieces of information, in which we integrate laboratory-based life history data and site specific climate records. Thus, we present a general outline

of our approach in a way providing a generalized framework of the technique. Finally, we demonstrate the applicability of the model by estimating environmental stochasticity arising from temporal and/or spatial temperature fluctuations on the springtail *Folsomia candida* (Willem), an ecologically important soil arthropod, frequently used as a model organism in ecotoxicology.

3.2 Theory

3.2.1 Tuljapurkar's Approximation in nutshell

Tuljapurkar's analytical method for estimating environmental stochasticity is based on a set of environment-dependent deterministic matrix population models and an environmental transition probability matrix. In the matrix model, the state of a population is given by a vector $N(t) = [N_1(t), \dots, N_n(t)]^T$, where $N(t)$ is the total populations size (number of individuals) at time t , $N_i(t)$ represents the number of individuals in age class i at time t and the superscript T indicates the transpose of vector [6]. This vector is projected from time t to $t + 1$ by a projection matrix \mathbf{A} :

$$N_{t+1} = \mathbf{A}N_t \quad (3.1)$$

where \mathbf{A} represents the projection matrix constructed from individual vital rates a_{ij} , while N_t and N_{t+1} represent the total number of indi-

viduals at time t and $t + 1$, respectively. The effect of environmental stochasticity on the population can be included by altering the vital rates, a_{ij} , in Equation 3.1 as a function of environmental conditions resulting in a set of deterministic environment-dependent projection matrices. Based on these environment-dependent projection matrices, Tuljapurkar suggested an analytical method for estimating the stochastic population growth rate [1, 11] and its variance. In its simplified form, the method expressed mathematically as follows ([2]; Equation 14.65 - 14.67):

$$\log \lambda_s \approx \log \lambda_1 - \frac{\tau^2}{2\lambda_1^2} + \frac{\theta}{\lambda_1^2} \quad (3.2)$$

where λ_s is the stochastic population growth rate, λ_1 is the dominant eigenvalue of the mean projection matrix (mathematically expressed in Equation (3.4), τ^2 is the measure of effect of temporal variability of the environment on the population dynamics (mathematically expressed in Equation 3.3a), θ is the effect due to autocorrelation (mathematically expressed as in Equation 3.3b):

$$\tau^2 = (v_1 \otimes v_1)^T C_0 (w_1 \otimes w_1) \quad (3.3a)$$

$$\theta = \sum_{j=2}^m (v_1 \otimes v_1)^T \left(\sum_{h=1}^{\infty} \left(\frac{\lambda_j}{\lambda_i} \right)^{h-1} C_h \right) (w_j \otimes w_1) \quad (3.3b)$$

λ_j in Equation 3.3a represents the dominant eigenvalue of the projection matrix for j 's environmental state, while v_j and w_j in Equation 3.3a, 3.3b represent the corresponding left and right eigenvectors, respectively the superscript T represents transpose of vectors; C_o is the covariance of the matrix elements and h is the environmental states. Tuljapurkar and Haridas [11] presented the step by step procedure for evaluation of τ^2 and θ . Let $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$ represent the set of environment-dependent projection matrices and P_1, P_2, \dots, P_N be the probabilities of occurrence of each matrix in every projection time; then the mean projection matrix \bar{A} can be evaluated as follows:

$$\bar{A} = \sum_{i=1}^n P_i \mathbf{A}_i \quad (3.4)$$

Thus, λ_1 represents the dominant eigenvalue of the mean projection matrix of Equation 3.4.

3.2.2 Parameter estimation and data requirements

As presented above, the environmental stochasticity is expressed using λ_s and σ^2 which can be calculated using Equations 3.2, 3.3a and 3.3b. Basically, these equations require: (1) demographic data for different environmental states that could be used for the estimation of the environment-dependent projection matrices $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$ (2) probability projection matrix P_{ij} , and (3) an environmental proba-

bility vector P_1, P_2, \dots, P_N which represents the probabilities of occurrence of projection matrices $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$.

For this, first we need demographic data for different temperature ranges that could be used for the estimation of the temperature-dependent projection matrices $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$. In case there are experimental data and/or empirical studies on individual vital rates at different temperatures, we can use these data for deriving temperature-dependent projection matrices. There are also readily available empirical models (e.g. [12]) for estimating vital rates as a function of temperature. Considering the short life history of the organisms we focus on, it is also possible to collect data at different temperature conditions and parameterize these empirical models (cf. [13–16]). Temperature dependent vital rates that can be derived from experimental data and/or empirical studies provide us with temperature ranges between which the vital rates are significantly different. We can use these temperature ranges to determine the number of projection matrices, while the vital rates could be used for developing and parameterizing the projection matrices.

The classification of the vital rates into different temperature regimes or ranges is accompanied by an environmental probability vector $P = [P_1, P_2, \dots, P_N]^T$ which represents the probability of occurrence of each temperature regime. This probability vector enables us to ran-

domly sample projection matrices from a set of temperature ranges under consideration. The novelty of our work lies in the technique we used to estimate this probability vector. It is based on discrete environmental states derived by using the Markov Chain approach. The first and most important information required in deriving this Discrete State Markov Chain is the estimation of the elements of the probability projection matrix P_{ij} , which represents the probability of occurrence of state i at time $t + 1$ given state j at time t . We used actual annual climate data records to derive and estimate P_{ij} . It needs to be mentioned here that the climate data should be (i) specific for (or near to) the study site under consideration, (ii) specific for the media in which the organism lives, and (iii) the records should be preferably daily. Once we have the temperature data, the next step is to divide the year into equal periods that are equivalent to the projection interval of our matrix model, and calculate the mean temperature for each period of time. We found a one week projection matrix, and dividing the annual temperature records into 52 weeks averages is mathematically tractable. However, in setting the length of the projection interval, care should be taken regarding the biology of the organism under study and all other issues related to development and parameterization of matrix models stated in the literature (cf. [2]). The mean temperature value evaluated for a specified pro-

jection time (that is weekly mean temperature in our case) can fall into one of the temperature ranges established earlier for the species of interest. Thus, we can estimate the probability of each projection time falling into each temperature range under consideration. By taking temperature records for a number of years, we estimated the temporal correlation of temperature. All that information enabled us to determine P_{ij} ; thus the probability of occurrence of each temperature range i at time t is given by:

$$P_{it} = [P_{ij}]^t \times P_{i0} \quad (3.5)$$

where P_{it} is the environmental probability vector i at time t , P_{ij} is the Discrete State Markov Chain based finite number of environmental states, and P_{i0} is the environmental probability vector i at the start of the simulation (i.e. $t = 0$).

3.3 Case study

The aim of the case study is to illustrate the step by step procedures of our approach in applying Tuljapurkar's method for estimating environmental stochasticity for short-lived organisms. We demonstrated the applicability of the method by considering the effect of temporal variation of temperature on the population dynamics of *Folsomia candida*. We used both unpublished life history data from our laboratory

and published data to investigate the temperature dependence of vital rates in *F. candida*. Eight-week observations of hatching time, growth rate and egg production at three temperatures (15, 20, and 25°C) were used to refine previously reported temperature dependence of vital rates of the organism (the experimental design and data analysis briefly described in Section 3.3.1). Five years (2006 to 2010) daily temperature data on soil surface and at 5 cm depth obtained from the Gaik-Brzezowa Research Station (Wieliczka Foothills) of the Institute of Geography and Spatial Management, Jagiellonian were used to characterize the temperature condition of the study area.

3.3.1 Experimental study

As explained above, data for this case study are partially obtained from the life history data we collected at three temperature conditions; 15, 20 and 25 °C. The experiment was conducted using a laboratory culture of *F. candida*, which had been kept for more than 10 years at the Institute of Environmental Sciences of Jagiellonian University, Poland. The animals were maintained as stock cultures in plastic boxes filled with moist plaster of paris, mixed with charcoal, at a constant temperature of 20 °C and with dried baker's yeast (Dr. Oetker) as food.

We explored the effect of temperature on the hatching time, matu-

urity time, growth and reproduction. For this, we designed three sets of experiments. In the first set, we observed the embryonic period of freshly laid egg at the three temperature conditions. In the second set, we monitored juveniles at the three temperature conditions and recorded time for the first reproduction and further monitored reproduction rate. In the third set, we measured the body size of the organism at the three test temperatures. Both experiments were carried out in plastic containers of different sizes with plastic screw top lids and filled with moist plaster of paris. The experiments were conducted in three climate chambers kept constant at 15, 20 and 25 $^{\circ}\text{C}$. Prior to the experiments, adult animals were transferred to plastic boxes ($\phi = 10\text{ cm}$) with plastic screw top lids and filled with moist plaster of paris to lay eggs.

In the first experiment, equal numbers of 20 freshly laid eggs were transferred to three plastic boxes ($\phi = 3.5\text{ cm}$). The three plastic boxes were randomly assigned to each of the three climate chambers and regularly monitored for hatching time. For the second experiment, 30 freshly hatched juveniles were transferred to 15 plastic boxes ($\phi = 5\text{ cm}$). Five plastic boxes, each containing two individuals, were randomly assigned to each of the three climate chambers and regularly monitored for eight weeks. In this experiment, we recorded the time when the juveniles start laying eggs. The plastic boxes were

monitored every week for reproduction using microscope. When eggs or juveniles were observed, the individuals were transferred to new test containers with the same conditions, and observations continued. The eggs and/or juveniles in the old test containers were photographed using a high-resolution photographs (pictures were taken using Canon EOS 1000D with 60 mm Macro lens) and the offspring were counted using ImageJ (v.1.47p); (National Institute of Health, USA, <http://imagej.nih.gov>) and manually. In the third experiment, body length at three temperature conditions was observed over eight weeks. For this, 30 freshly hatched juveniles were transferred to three plastic boxes ($\phi = 15$ cm), 10 individuals per each plastic box, and the plastic boxes were randomly assigned to each of the three climate chambers. Body length of the individuals were observed over eight weeks. Digital image processing equipment was used to record body lengths (recorded every day for the first 3 weeks and every 2 - 4 days thereafter until the end of the experiment). Body lengths (i.e., the distance from the posterior end of the abdomen to the anterior end of the head between the antennae) were measured using the free image analysis software ImageJ (v.1.47p).

3.3.2 Structure of the matrix model

As presented above, the first step of our approach was to estimate the different temperature ranges in which the vital rates are significantly different, and parameterizing the projection matrices accordingly. For this, we needed to formulate the projection matrix first. The first step in formulating the matrix model was to select the structure of the projection matrix (age, stage or size classified) and to determine the appropriate number of age/stage classes. Temperature significantly affects the longevity of *F. candida* (discussed in the following sections), which makes the age based projection matrix difficult to analyze as we would have differently sized matrices for the different temperature regimes. However, the life history of *F. candida* can be easily divided into three stages: eggs (embryos), juveniles and adults. Thus, we used the following three stage based model:

$$\begin{bmatrix} P_1 & 0 & F_3 \\ G_1 & P_2 & 0 \\ 0 & G_2 & P_3 \end{bmatrix} \times \begin{bmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \end{bmatrix} = \begin{bmatrix} N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} \quad (3.6)$$

where P_i is the probability of remaining in the same stage, G_i the probability of moving to the next stage, F_i the number of eggs produced per individual, and N_{it} and N_{it+1} represent the number of

individuals in the different developmental stages at time t and $t + 1$ respectively. As suggested by Caswell [2], P_i and G_i can be calculated as follows:

$$P_1 = \sigma_1(1 - \gamma_1) \quad (3.7a)$$

$$G_1 = \sigma_1\gamma_1 \quad (3.7b)$$

$$P_2 = \sigma_2(1 - \gamma_2) \quad (3.7c)$$

$$G_2 = \sigma_2\gamma_2 \quad (3.7d)$$

$$P_3 = \sigma_3 \quad (3.7e)$$

where σ_1, σ_2 and σ_3 are the probabilities of survival of embryos, juveniles and adults, respectively, γ_1 is the fraction of embryos surviving and moving to the juvenile stage, and γ_2 is the is the fraction of juveniles surviving and moving to the adult stage. γ_1 and γ_2 can be determined from growth rate parameters of eggs, τ_1 , juveniles, τ_2 , and projection time, t , as follows:

$$\gamma_1 = \frac{t}{\tau_1} \quad (3.8a)$$

$$\gamma_2 = \frac{t}{\tau_2} \quad (3.8b)$$

The reproduction rate F_3 , expressed as the number of eggs produced per individual per projection time (week in our case), can be deter-

mined based on data collected on the fertility of *F. candida*. In our simulations we used a projection interval of one week.

3.3.3 Influence of temperature on vital rates

Effect of temperature on one or more of the vital rates results in a temperature dependent projection matrix. Temperature dependence of vital rates of *F. candida* was reported in a number of studies. Particularly, temperature dependence of rate related vital rates, i.e. reproduction and growth, were reported by many authors (e.g. [16–22]). Our eight weeks observations also clearly show the influence of temperature on the growth and reproduction of *F. candida*. From our study, we found that at 15 °C, *F. candida* begins ovipositing at the age of 30-39 days, while it takes 18-25 days at 20 °C and 16-21 days at 25 °C. The average time it takes the eggs to hatch at 15, 20 and 25 °C has been estimated to 19, 11 and 7 days, respectively. Figure 3.2 shows these individual rates for three temperature conditions.

There are also studies aimed at relating the survival of *F. candida* to temperature. Snider and Butcher [17] studied the age specific survival and longevity of *F. candida* at three temperatures (15, 21 and 26 °C) and found that higher temperature reduces individual life span. Contrary to that, we did not find significant difference in the age-specific survival rates of *F. candida* among the three temperatures

used in our study. Therefore, we assumed that survival of adults is less temperature dependent, which is realistic within a wide temperature range [15].

According to previous studies, hatching success of the eggs is temperature dependent. At 10 °C only 12% of eggs hatch and this percentage increases with temperature and reaches maximum value of 94.5% at 21 °C and starts declining at 26 °C [23]. Thus, for the survival of embryo stage, we derived a temperature dependent function for the survival rate of embryos as explained below.

3.3.4 Derivation of the temperature dependent vital rates

We used the first order Taylor's series approximation to derive a functional relationship between temperature and rate related vital rates (i.e., development rate and egg production rate) of the organism. Mathematically the method is expressed as follows:

$$Z(T) = Z(T_0) + (T - T_0) \frac{dZ}{dT} \Big|_{T=T_0} \quad (3.9)$$

Where $Z(T)$ is development or reproduction rate at temperature T , $Z(T_0)$ development or reproduction rate at known reference temperature T_0 , and the differential equation at the right hand side is the 1st order derivative of the $Z(T)$ and evaluated at the reference tempera-

ture, T_0 . We considered T_0 to be the minimum temperature at which growth or producing of eggs starts. Thus, we can take $Z(T_0) = 0$ and Equation 3.9 can be simplified to:

$$Z(T) = (T - T_0) \frac{dZ}{dT} \Big|_{T=T_0} \quad (3.10)$$

Equation 3.10 is equivalent with the linear equation (with the derivative as slope and $-T_0 \frac{dZ}{dT} \Big|_{T=T_0}$ as intercept) used by Choie et al. [13] for expressing the relationship between development rate (Z) and temperature (T) for collembolans. Stam et al. [16] also found a linear relationship between the development rate of eggs and juveniles of *F. candida* and temperature. However, the accuracy of Equation 3.10 can be compromised as the temperature T gets higher (i.e., far from the minimum threshold temperature, T_0). Thus, instead of using a constant intercept and slope, we applied a spline linear interpolation between consequent temperatures with different intercept of $Z(T_{i-1}) - T_{i-1} \frac{dZ}{dT} \Big|_{T=T_0}$ and slope of $\frac{dZ}{dT} \Big|_{T=T_{i-1}}$. The spline linear interpolation relationship is used to estimate a set of intercepts. The reproduction rate of *F. candida* increases with temperature up to 21 °C and then gradually decreases with temperature [16, 17]. The maximum temperature for egg production is estimated to be between 28 and 30 °C [23]. Thus, a negative slope is used for the reproduc-

tion rate for the temperature range between 22 and 30 °C. All the aforementioned primary and published life history data were used to estimate the minimum temperature threshold (T_0) and parameters of the series of linear spline interpolation equations for both development rate and production of eggs.

The time required to complete embryo and juvenile stages at different temperatures, together with temperature-dependent eggs hatchability [23] and the temperature-dependent survival [17], were used to estimate the temperature dependent survival of the eggs and juveniles. Table 3.1 summarizes the estimated values of the parameters used for deriving the temperature dependence of the vital rates together with the data sources and/or method applied, while Figure 3.1 elucidates the functional relationship between the rates and temperature.

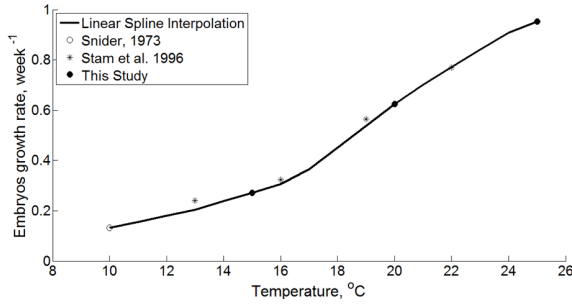
3.3.5 Parameterizing temperature dependent projection matrix

From Table 3.1 it is clear that one or more of the vital rates of *F. candida* differ between the four temperature regimes: ≤ 0 °C (no juvenile survival), 0–5 °C (no juvenile growth), 6–8 °C (no egg development), 8–10 °C (no egg production). Further we used the data in Table 3.1 and Equation 3.10 to identify temperature ranges with a significant difference in one or more of the vital rates. Accordingly, we

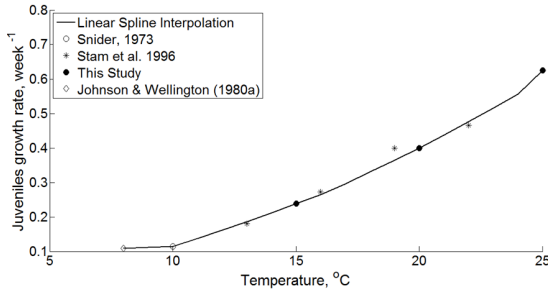
Table 3.1: Important life history parameters and their threshold temperatures. Threshold temperatures for egg development, juvenile development and egg production are expressed in degree days, DD^{-1} , which represents the organism would have to accumulate in order to complete a life stage.

Parameter	Value	Data sources and Estimated from
Minimum temperature for egg development	$\approx 8^{\circ}C$	[16], [17], [19] and Equation 3.10
Minimum temperature for embryo survival	$\approx 5^{\circ}C$	[17]
Maximum temperature for egg development	$28^{\circ}C$	[22]
Minimum temperature for juvenile growth	$\approx 5^{\circ}C$	[16], [17], [19], this study and Equation 3.8b
Minimum temperature for juvenile survival	$\approx 0^{\circ}C$	Assumed
Minimum temperature for egg production	$\approx 10^{\circ}C$	[18], [17], [21], this study and Equation 3.10
Maximum temperature for egg production	$\approx 10^{\circ}C$	Study on Collembolan ([13])
$\frac{dZ}{dT} _{T=8^{\circ}C}$: (Egg development)	$\approx 0.0027 DD^{-1}$	[16], [17], [19] and Equation 3.8a
$\frac{dZ}{dT} _{T=5^{\circ}C}$: (Juvenile development)	$\approx 0.018 DD^{-1}$	[16], [17], [19] this study and Equation 3.8b
$\frac{dZ}{dT} _{T=5^{\circ}C}$: (egg production)	≈ 0.018 eggs DD^{-1}	[18], [17], [21], this study and Equation 3.10

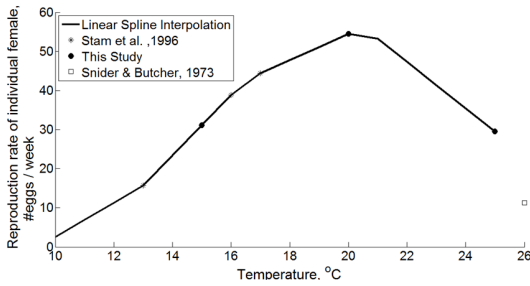
identified seven additional temperature regimes (in °C): 10-12 (reproduction starts but hatchability is at its lowest level), 12-15, 16-18, 19-22 (reproduction rate is maximum), 23-25 (reproduction rate declines with temperature), 26-30 (the maximum tolerance temperature for reproduction and egg development), and 30-35 (no egg production). This classification resulted a total of 11 temperature regimes. These 11 temperature ranges were further refined, based on actual temperature conditions of the study area, as follows. As the minimum and maximum weekly temperatures at 5 cm deep in grass soil is -0.7 and 25 °C (Figure 3.3(a) and Figure 3.3(b)), the temperature ranges above 25 °C were excluded from our analysis. Therefore, the 11 temperature regimes that influence the vital rates of *F. candida* differently from each other were further re-grouped and reduced to eight regimes. These eight temperature regimes represent not only the classification of the temperature dependent vital rates, but also the temperature classification of the study area in terms of the life history of the species. Table 3.2 summarizes the eight temperature regimes used finally in the model and the estimated values.



(a)



(b)



(c)

Figure 3.1: Temperature dependence of vital rates: (a) embryonic growth rate, week⁻¹, (b) juveniles growth rate, week⁻¹ and (c) adults reproduction rate, number of eggs per week per female. Data sources for deriving the linear spline interpolation equations are indicted in the legends.

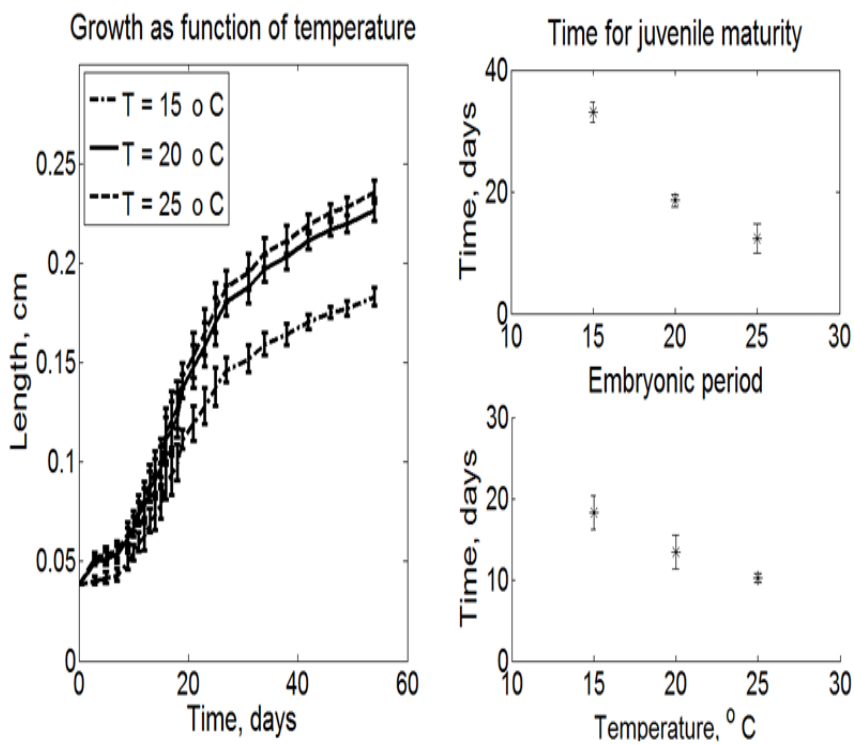


Figure 3.2: Growth, juvenile maturity time and egg hatching time for three temperature conditions. Bars indicate the standard errors

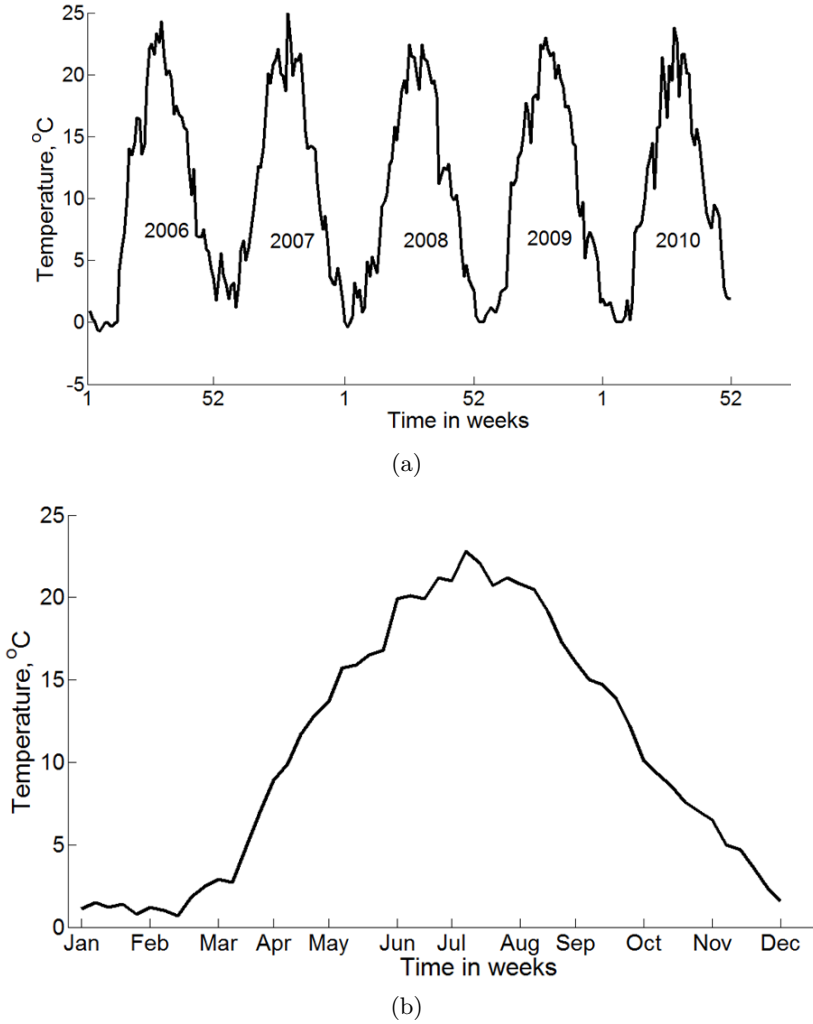


Figure 3.3: Weekly mean temperatures for the Gaik-Brzezawa Field Station in the period 2006-2010 (a) and average weekly temperatures for the whole 5-year period (b). Week Numbers in the x-axis (b): the first week of January represented as week 1, while week 52 is the last week of December.

Table 3.2: Summary of temperature regimes influencing the vital rates of *F. candida*. σ_i s are expressed per week; τ_1 and τ_2 are in weeks and F_3 is the number of eggs per female per week.

Vital rates	Temperature ranges (in °C)							
	< 0	0 - 5	6 - 8	9 - 11	12 - 14	15 - 18	19 - 22	23 - 25
Embryos survival, σ_1	0	0	0.09	0.12	0.52	0.87	0.93	0.87
Embryos growth, τ_1	∞	∞	∞	7.6	4.9	3.1	1.5	1.1
Juveniles survival, σ_2	0	0.7	0.83	0.84	0.9	0.9	0.96	0.96
Juveniles growth, τ_2	∞	∞	9.2	8.8	5.4	3.6	2.4	1.8
Adult survival, σ_3	0.65	0.65	0.65	0.75	0.73	0.68	0.65	0.6
Adult repro- duction, F_3	0	0	0	0.7	12	40	64	30

The eight temperature regimes were used to derive and parameterize eight distinct projection matrices. Accordingly, the set of temperature dependent vital rates in Table 3.2 were used to calculate the parameters of the projection matrices using Equations 3.7 and 3.8. Table 3.3 summarizes the eight temperature dependent parameters

of the three stage projection matrix of Equation 3.1

Table 3.3: Temperature dependent vital rates used for the parameterization of the projection matrix \mathbf{A} (Equation 3.1) for the eight temperature regimes.

Temperature range	Parameters					
	P_1	P_2	P_3	G_1	G_2	F_3
< 0	0	0	0.65	0	0	0
0 - 5	0	0.70	0.65	0	0	0
6 - 8	0.09	0.74	0.65	0	0.089	0
9 - 11	0.104	0.745	0.75	0.016	0.097	0.7
12 - 14	0.414	0.73	0.73	0.106	0.167	12
15 - 18	0.61	0.650	0.68	0.288	0.251	40
19 - 22	0.31	0.63	0.65	0.624	0.33	64
23 - 25	0.079	0.427	0.60	0.791	0.524	30

3.3.6 Estimating the Markov Chain environmental probability matrix

We used the actual temperature data for deriving the environmental probability matrix P_{ij} for the eight temperature regimes derived above. For this purpose, the five years daily temperature data obtained from our study site is re-calculated into mean weekly temperatures to fit our one-week projection matrix model. Figure 3.3(a) shows the weekly temperatures from 2006 to 2010, while Figure 3.3(b) presents average weekly temperatures for the whole period for the study area.

The five years temperature data were used to derive a time dependent continuous function: assuming no significant year to year climate change in the study area, we generated a weekly set of temperature data using Equation 3.11.

$$T(t) = \alpha \cos \beta(t - \gamma) + \delta \quad (3.11)$$

where $T(t)$ is the temperature in $^{\circ}\text{C}$ at time t (in weeks 1-52), α , β , γ , δ are constants. The values of the constants (together with their 95% CIs) are given in Table 3.4, while Figure 3.4 shows the data points (open circles) and the fitted function Equation 3.11 (solid line).

Based on the average weekly temperatures, each week was assigned

Table 3.4: Parameter estimates of Equation 3.11 and goodness to fit

Parameters	Values (95%CI)
α	10.44 (10.13,10.75)
β	0.1206 (0.1202, 0.121)
γ	-22.59 (-23.16, -22.02)
δ	10.71 (10.49, 10.93)
Goodness of the data fit: R^2 is 0.9456	

to one of the eight temperature ranges specified above, while the five years of weekly data and the data generated using Equation 3.11 were used to derive the Markov chain-based environmental states, P_{ij} . In

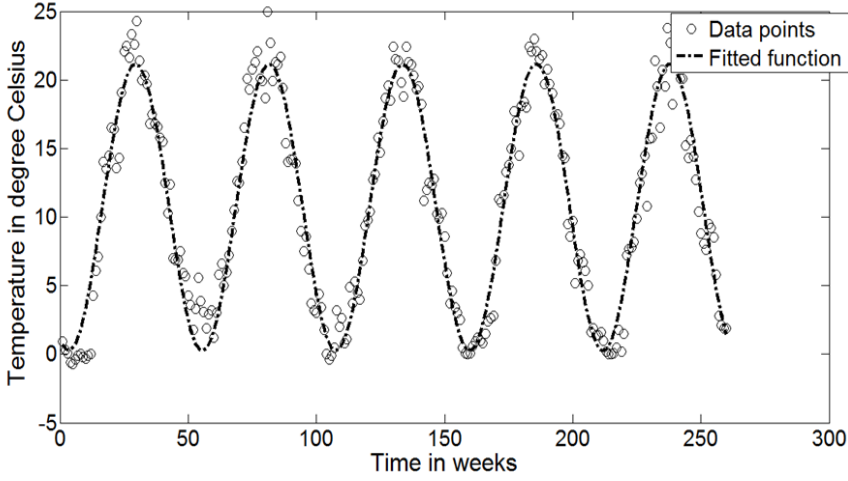


Figure 3.4: The continuous function (Equation 3.10; solid line) fitted to actual weekly mean temperatures (open circles) for the Gaik-Brzezowa Field Station in the period 2006-2010.

brief, P_{ij} is a probability of starting the projection from temperature range j at time t , and ending up at temperature range i at time $t+1$. Temperature range i can be either the temperature range j (i.e., the same state as in time t) or any other from among the remaining seven ranges. This environmental transfer projection matrix is constructed by using a random walk model. If we consider the environment stays in the current state with probability q , the probability of moving to the other seven states is $\frac{1-q}{7}$. However, the probability of falling into any temperature range is not equal; for example, during the winter season, the probability of entering in temperature range 19-

22 °C is null, and the highest probability was for temperatures < 5, etc. Therefore, we introduced a weight factor W_j which describes the weight of the seven environmental states. Accordingly, this can be described by a random walk model, with transition matrix satisfying:

$$P_{ij} = \begin{cases} q & \text{if } i = j \\ W_j(\frac{1-q}{7}) & \text{if } i \neq j \end{cases} \quad (3.12)$$

The weekly average climate data were used to estimate q 's and W_j 's. The environmental transfer probabilities (P_{ij}) for the study area are given in Table 3.5 as a column Markov chain probability matrix. Once

Table 3.5: Column based Markov chain discrete state probability matrix elements of the study area. The first row and column represent the temperature ranges. The transfer probabilities $P_{i,j}$ s is given as 8 by 8 square matrix

	< 0	0 - 5	6 - 8	9 - 11	12 - 14	15 - 18	19 - 22	23 - 25
< 0	0.30	0.10	0	0	0	0	0	0
0 - 5	0.55	0.69	0.40	0.05	0.01	0	0	0
6 - 8	0.10	0.15	0.40	0.30	0.05	0.05	0	0
9 - 11	0.05	0.06	0.15	0.38	0.15	0.05	0.03	0
12 - 14	0	0	0.02	0.10	0.5	0.10	0.05	0.05
15 - 18	0	0	0.03	0.10	0.15	0.60	0.17	0.10
19 - 22	0	0	0	0.07	0.10	0.15	0.6	0.45
23 - 25	0	0	0	0	0.04	0.05	0.15	0.40

we determined the transfer probabilities P_{ij} , we could use Equation

3.5 for estimation of the environmental probability vector, P_{it} - the 8 by 1 column vector at time t , in which rows represent the probability of occurrence of the eight temperature ranges. However, to use Equation 3.5 we need an initial environmental vector P_{i0} , which represents the environmental probability vector at the start of projection (i.e., the temperature range at $t = 0$). We can assign P_{i0} based on the week (which determines the temperature range) and make the projection for the specified time, t . For long-term simulations, we can assign any of the column vectors from P_{ij} as initial environmental probability vector P_{i0} ; hence for large t , P_{it} in Equation 3.5 is constant irrespective of P_{i0} . Accordingly, we tried all the eight columns of P_{ij} (i.e. from the 2nd to the 9th column) in Table 3.5 as initial vector P_{i0} and evaluated Equation 3.5 for $t = 1000$ and we found a consistent probability vector, of P_{it} .

Table 3.6: The probability of occurrence of the eight weekly temperature regimes, P_{it} . The values represent the frequency at which each temperature regime is expected to occur in a year

Temperature < 0 range (°C)	0 - 5	6 - 8	9 - 11	12 - 14	15 - 18	19 - 22	23 - 25	
P_{it}	0.04	0.279	0.146	0.103	0.077	0.145	0.154	0.056

3.3.7 Including stochasticity: estimating λ_s and σ_e^2

The eight temperature dependent matrices constructed using the parameters from Table 3.3 and their probability of occurrence, P_{it} , from Table 3.6 were used to estimate the stochastic population growth rate, $\log(\lambda_s)$, and σ^2 using Equation 3.2 and 3.3. For the sake of comparison, different deterministic population growth rates were also evaluated. All calculations were performed with a program code developed in MATLAB (Version 8.0.0.783, R2012b).

3.4 Results

3.4.1 Temperature dependent deterministic intrinsic population growth rate

The determined asymptotic population growth rates λ for the eight temperature ranges are given in Table 3.6. The deterministic population growth part of Tuljaparkur's analytical equation (λ_1 in Equation 3.2) has been estimated to be 1.414. This value is less than the deterministic population growth rate estimated for the temperature range of 15-18 °C but higher than the temperature range of 12-14 °C. On the other hand, the mean annual weekly temperature of the soil in the study area is estimated to be 11.7 °C; at this temperature λ was estimated to be 1.087.

Table 3.7: Deterministic population growth rates of the eight temperature regimes

Temperature	< 0	0 - 5	6 - 8	9 - 11	12 - 14	15 - 18	19 - 22	23 - 25
range ($^{\circ}\text{C}$)								
λ	0.65	0.70	0.74	0.787	1.236	2.072	2.897	2.693
$r_{int},$	-0.4	-	-0.3	-0.2	0.21	0.73	1.06	0.99
(week^{-1})		0.36						

3.4.2 Estimating effect of environmental stochasticity

Using Tuljapurkar’s analytical equations (Equation 3.2 and Equation 3.3), we estimated the stochastic population growth rate $\lambda_s = 1.057$ and the variance $\sigma_e^2 = 0.367$. The results from the case study indicate that the stochastic population growth rate ($\lambda = 1.057$) is lower than the deterministic population growth part of Tuljaparkur’s analytical equation ($\lambda_1 = 1.414$) in Equation 3.2. Thus, estimating population growth rate in a variable environment without taking into account environmental stochasticity may overestimate population viability. The higher σ_e^2 in the stochastic environment indicates the significance of the effect of the seasonal temperature fluctuations on the fitness of *F. candida* population. The variation and co-variation among the vital rates (the terms inside of the summation in Equation 3.3a) is the main contributor for this higher value of σ_e^2 .

3.5 Conclusions

The approach presented in this paper proved to be useful in estimating effects on population dynamics of stochasticity arising from environmental fluctuations. Although it has been tested on one case study only, we assume that it will equally utile for any species, in any environment (e.g., terrestrial or aquatic), and for any type of environmental factor. The ease of data accessibility for parameterizing and execution the sub-models is the important feature of the approach. Detailed biological data are often available for species used as standard ecological and ecotoxicological test organisms even at different environmental conditions. Considering their short lifespan, it is even possible to conduct full and partial life table studies at different environmental conditions. Seasonal climate data records are easily available from meteorological stations around the world. As environmental fluctuations are derived from actual climate records, the model can be also used to predict effects of the future environmental changes (for example global warming) on the dynamics of short-lived organisms. Another possible area of application includes, but is not limited to, assessing and predicting combined effects of chemicals and temporal environmental fluctuation on populations in their natural environment under chemical stress or predicting long term effects of future local, regional and global environmental changes on the dy-

namics of vulnerable species. Particularly, it may have a significant role in the field of ecotoxicology and ecological risk assessment.

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Chapter 4

Stochastic density dependent matrix model for extrapolating individual-level effects of chemicals to the population: Case study on effects of Cd on *Folsomia candida*

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Abstract

The extrapolation of individual-level effects observed in standard bioassays to the population level is one of the major challenges in ecotoxicology and ecological risk assessment of chemicals. Most of the information about ecotoxicity of chemicals is currently derived from laboratory ecotoxicological tests in which individual-level effects (e.g., the effects on individual life span, fertility, and body growth) of toxic chemicals are measured. However, the risk of chemicals at the population level may be strongly affected by stochastic events that can arise from population structure, density dependence, timing of exposure and environmental fluctuations which are not considered in laboratory experiments. In this study, we present a matrix model framework for assessing the ecological risks of chemicals on populations in the field by accounting for density dependence and environmental stochasticity arising from seasonal environmental fluctuations. As an illustration, we present a case study that predicts effects of cadmium (Cd) on *Folsomia candida* populations by considering environmental stochasticity arising from temporal and/or spatial temperature variation. The model results show that extrapolating individual-level effects of Cd to a higher level of biological organization without considering environmental variability underestimates effects of chemicals on population growth rate. Chemicals can also reduce the popu-

lation size at its carrying capacity. The latter phenomenon arises from different effects of toxicants on different age/stage classes combined with natural demographic processes. One important and useful population-level endpoint that can combine all these effects (i.e. environmental variability, chemical-specific toxicity, and density dependence) is the mean extinction time. Our model simulation results show that the mean extinction time of the *F. candida* population exposed to 1000 mg Cd/kg soil can be reduced by 40% compared to the control population. Nevertheless this significant reduction will not lead to extirpation of the population as the estimated extinction time is very long: over 3000 years. Based on these results, a population of *F. candida* exposed to this concentration of Cd may not be considered to be endangered. However, other stresses (like habitat fragmentation, food limitation, predation, disease, etc.) can additionally influence the mean extinction time.

Key words: ecological risk assessment; population models; environmental variability; mean extinction time; dose-response models; extrapolation; parametric matrix method

4.1 Introduction

In standard ecotoxicological tests (bioassays), effects of chemicals are measured for selected species at the individual level. The most frequent endpoints are changes in body size, reproduction rate and short-term survival. These state variables are easy to measure and the data are inexpensive to produce, hence the bioassays are widely used for chemical risk assessments. The relevance of these short-term bioassays in view of the functioning of populations and ecosystems has been, however, questioned by a number of authors (cf. [1–5]). Moreover, the use of individual-level endpoints, which are derived from these individual-level measurements, like the No-Observed Effect Concentration (NOEC), as an index for ecological risk assessment of chemicals, has also been criticized (e.g. [6–8]). Regulations, policies, directives and guidance documents frequently discuss the need for ecological risk assessments to consider risks to populations, and not simply to individual organisms or organism-level life-history traits. Therefore, ecological impacts of toxicants have been increasingly assessed with the aid of population-level methods [1, 2, 9].

This shift from studying effects on individuals to populations is gaining momentum with increasing emphasis on population-level assessments [1, 10]. Furthermore, directives and guidelines request risk assessment at higher levels of biological organization [11, 12]. Neverthe-

less, information on the ecotoxicity for population-level risk assessments is still derived from laboratory individual-level bioassays, which are conducted under constant and, presumably, favorable conditions. However, in their natural environment, organisms rarely experience optimal conditions, and these conditions are rarely constant. On the contrary, organisms are forced to cope with either suboptimal or severe environmental stress for most of their life spans [13, 14]. These added environmental stressors may or may not alter the effects of chemical contaminants in comparison to laboratory tests performed under controlled and optimal conditions. Risk assessment procedures therefore typically include safety (or uncertainty) factors in order to ensure conservative estimates of environmental concentrations that minimize risks posed by chemicals to organisms in the natural environment [15].

These uncertainty factors usually lack a scientific basis and may underestimate or overestimate actual environmental effects [16]. On the other hand, it is technically impossible as well as economically too expensive to experimentally assess all possible interactions between different environmental conditions and chemicals.

This lack of realism in standardized bioassays makes the interpretation and extrapolation of observed individual-level effects to the population level uncertain. For example, Forbes and Calow [17] evaluated

effects of toxicants at both individual and population levels and found an inconsistent and unpredictable relationship between the two. The authors also found that effects of a toxicant on population growth rate (r) may be smaller, equal to or larger than effects on individual life-history traits contributing to it.

In a recent meta-analysis, Laskowski et al. [18] reviewed and analyzed a range of significant effects of natural environmental conditions on toxicant effects in animals. Heugens et al. [14] reported the influence of variable and suboptimal environmental conditions on the outcome of toxicity tests. Besides effects of natural physico-chemical stressors, interactions between toxicants and density dependence, and their influence on population dynamics have gained interest among ecotoxicologists [4, 19–25].

Therefore, understanding and including intra-population and extra-population ecological factors that can modify effects of toxicants at individual and population levels is a key step in developing tools for extrapolating the results of laboratory toxicity tests to populations living in natural environments. Matrix population models can be used to investigate the dynamics of age/stage classified populations [26, 27] and can estimate many characteristic population endpoints, such as asymptotic population growth rate, generation time, stable population structure, and reproductive values [28].

In this work, we present a general matrix population model framework for assessing the ecological risks of chemicals on populations in the field by accounting for density-dependence and environmental stochasticity arising from seasonal environmental fluctuations. As an illustration, we provide a case study that predicts the effect of cadmium (Cd) on *Folsomia candida* populations by considering environmental stochasticity arising from temporal and/or spatial temperature variation.

4.2 Materials and methods

4.2.1 General model structure

The general structure of the model is based on an age/stage based matrix. In the matrix model the state of a population is given by a vector $N(t) = [N_1(t), \dots, N_n(t)]^T$, where $N(t)$ is the total population size (number of individuals) at time t , $N_i(t)$ represents the number of individuals in age/stage class i at time t and the superscript T indicates the transpose of vector [6]. This vector is projected from time t to $t + 1$ by a projection matrix \mathbf{A} :

$$N_{t+1} = \mathbf{A}N_t \tag{4.1}$$

where \mathbf{A} represents the projection matrix constructed from individual vital rates a_{ij} , while N_t and N_{t+1} represent the total number of

individuals at time t and $t + 1$, respectively.

This general age/stage based matrix model can be extended to model population-level effects of environmental stressors by including measured effects of a stressor on specific vital rates and by incorporating key ecological processes that can affect population dynamics (e.g. seasonal environmental variation and density-dependence in our case).

The framework of our model comprises five main elements: formulating the projection matrix, modelling effects of toxicants at the individual-level, estimating environmental stochasticity, modelling effects of density-dependence and eventually estimating overall population-level effects. The brief description of the methodological approach we applied to address these elements of the model is presented in the subsequent sections by considering the effect of Cd on *F. candida* populations.

4.2.2 *F. candida* as a standard bioassay species

F. candida is the most widely studied springtail species, both in terms of its ecotoxicology and life history, and it has been extensively used for toxicity testing [29]. They are easy to culture in the laboratory, and due to parthenogenetic reproduction, populations of one particular genotype can be obtained. Furthermore, an extensive amount of literature is available about the individual-level effects of toxicants

and environmental stressors on *F. candida*. The species is used as a model organism in the OECD guideline 232 (collembolan reproduction test in soil) as a representative organism for risk assessment of chemicals to soil invertebrates.

4.2.3 Stage-based matrix model

The first step in formulating a matrix model is to select the type and structure of the projection matrix (age, stage, or size classified) and to determine an appropriate number of age/stage classes. The life history of *F. candida* consists of three distinct stages: egg (embryo), juvenile, and adult, and therefore we used a three-stage based matrix model (Equation 4.2):

$$\begin{bmatrix} P_1 & 0 & F_3 \\ G_1 & P_2 & 0 \\ 0 & G_2 & P_3 \end{bmatrix} \times \begin{bmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \end{bmatrix} = \begin{bmatrix} N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} \quad (4.2)$$

where P_i is the probability of remaining in the same stage, G_i the probability of moving to the next stage, F_i the number of eggs produced per individual, and N_{it} and N_{it+1} represent the number of individuals in the different developmental stages at time t and $t+1$ respectively. As suggested by Caswell [26], P_i and G_i can be calcu-

lated as follows:

$$P_1 = \sigma_1(1 - \gamma_1) \quad (4.3a)$$

$$G_1 = \sigma_1\gamma_1 \quad (4.3b)$$

$$P_2 = \sigma_2(1 - \gamma_2) \quad (4.3c)$$

$$G_2 = \sigma_2\gamma_2 \quad (4.3d)$$

$$P_3 = \sigma_3 \quad (4.3e)$$

where σ_1 , σ_2 and σ_3 are the probabilities of survival of embryos, juveniles and adults, respectively, γ_1 is the fraction of embryos surviving and moving to the juvenile stage, and γ_2 is the fraction of juveniles surviving and moving to the adult stage. γ_1 and γ_2 can be determined from growth rate parameters of eggs, τ_1 , juveniles, τ_2 , and projection time, t , as follows:

$$\gamma_1 = \frac{t}{\tau_1} \quad (4.4a)$$

$$\gamma_2 = \frac{t}{\tau_2} \quad (4.4b)$$

The reproduction rate F_3 , expressed as the number of eggs produced per individual per projection time (one week in our case), can be determined based on data collected on the fertility of *F. candida*. In all simulations of population dynamics we used a projection time step

of one week.

4.2.4 Modelling the individual-level effects of chemicals

The individual-level effects of a chemical can be obtained using the dose-response relationship derived from bioassay test data. This relationship has played a major role in the history of toxicology [30]. A typical dose-response curve is sigmoid, and one example of such a curve is the log-logistic curve [31]. Accordingly, we used Equation 4.5 to describe this dose-response relationship.

$$y = C + \frac{D - C}{1 + \left(\frac{x}{EC_{50}} \right)^b} \quad (4.5)$$

Where:

y = proportional response of the endpoint—survival, growth rate or reproduction in this case x = toxicant concentration

C = the lower limit of σ_1 , σ_2 , σ_3 , γ_1 , γ_2 and F_3 (corresponds to the mean response at very high doses)

D = the upper limit of σ_1 , σ_2 , σ_3 , γ_1 , γ_2 and F_3 (corresponds to the mean response of the control)

b = slope (or shape factor), which describes the steepness of the concentration-response curve.

EC_{50} = concentration that results in a 50% response - the inflection

point of the sigmoid curve.

One of the advantages of using the curve described by Equation 4.5 is that the parameters are biologically meaningful [31]. The upper limit D corresponds to the mean response of the control, and the lower limit C is the mean response at very high doses (note that the lower limit is not necessarily zero). The parameter b determines the slope of the curve around the EC_{50} . The greater the value of b , the steeper the slope of the curve, and the more uniform the response of individuals. Accordingly, these bioassay derived responses should be included in the projection matrix in equation 4.2 by correcting the vital rates as a function of the toxicant concentration, x .

4.2.5 Effect of seasonal temperature fluctuations

Effects of seasonal temperature fluctuations are included in our model by estimating the environmental stochasticity arising from this variable. The usual practice for this is to formulate stochastic matrix population models of the form in Equation 4.2, under the assumption that the variation in vital rates among matrices is due to fluctuating environmental conditions, and estimate the stochastic population growth rate and its variance [26, 27, 32, 33]. Generally, stochastic matrix models can be formulated either by selecting whole observed deterministic matrices (estimated from the environment-dependent

vital rates) at random at each time step of a simulation or by selecting the vital rates at random from some statistical distribution [34]. Selecting vital rates at random from a statistical distribution is based on independent variation of matrix entries. This independent variation of matrix entries violates the correlation between vital rates. Caswell [26] also pointed out that developing stage-based stochastic matrix models by varying matrix entries independently can lead to unreasonable results, hence the column sums of the matrix that describes individual transitions (for example $P_1 + G_1$ and $P_2 + G_2$ in equation 4.2) can exceed unity. Thus, in this study, we used the former technique as it avoids these shortcomings of independent variation of matrix entries. In this method there are two distinct techniques: the "parametric matrix method" (PMM) and the analytical method proposed by Tuljapurkar [35]; see [33] for detail discussion of the methods. Here we used Tuljapurkar's analytical method, which is represented mathematically as:

$$\log \lambda_s \approx \log \lambda_1 - \frac{\tau^2}{2\lambda_1^2} + \frac{\theta}{\lambda_1^2} \quad (4.6)$$

where λ_s is the stochastic population growth rate, λ_1 is the dominant eigenvalue of the mean projection matrix (mathematically expressed in Equation (4.6), τ^2 is the measure of effect of temporal variability

ity of the environment on the population dynamics (mathematically expressed in Equation 4.7 a), θ is the effect due to autocorrelation (mathematically expressed as in Equation 4.7 b):

$$\tau^2 = (v_1 \otimes v_1)^T C_0 (w_1 \otimes w_1) \quad (4.7a)$$

$$\theta = \sum_{j=2}^m (v_1 \otimes v_1)^T \left(\sum_{h=1}^{\infty} \left(\frac{\lambda_j}{\lambda_i} \right)^{h-1} C_h \right) (w_j \otimes w_1) \quad (4.7b)$$

λ_j in Equation 4.7 a represents the dominant eigenvalue of the projection matrix for j 's environmental state, while v_j and w_j in Equation 4.7 a and 4.7 b represent the corresponding left and right eigenvectors respectively; the superscript T represents the transpose of vectors; C_o is the covariance of the matrix elements and h is the environmental states. Tuljapurkar and Haridas [36] presented the step by step procedure for evaluation of τ^2 and θ . The deterministic part of the population growth rate, λ_j in Equation 4.6, is the dominant eigenvalue of the mean projection matrix \bar{A} , which can be estimated from environmental-dependent projection matrices $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$ and their corresponding frequency vectors; P_1, P_2, \dots, P_N . This means that the projection matrix can be evaluated as follows;

$$\bar{A} = \sum_{i=1}^n P_i \mathbf{A}_i \quad (4.8)$$

Thus, λ_1 represents the dominant eigenvalue of the mean projection matrix of Equation 4.8.

As presented above, the environmental stochasticity is expressed using λ_s and σ^2 which can be calculated using equations 4.6, and 4.7. Basically, these equations require: (1) demographic data for different environmental states that could be used for the estimation of the environment-dependent projection matrices $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$ (2) probability projection matrix P_{ij} , and (3) an environmental probability vector P_1, P_2, \dots, P_N which represents the probabilities of occurrence of projection matrices $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$. Thus, first we need demographic data for different temperature ranges that could be used for the estimation of the temperature-dependent projection matrices $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$. The environmental probability vector $\mathbf{P} = [P_1, P_2, \dots, P_N]^T$ represents the probability of occurrence of the temperature ranges defined above. The probability vector enables us to sample a specific projection matrix randomly from those temperature ranges under consideration. The usual practice for estimating these parameters is to use field-based year to year environment-dependent life-history data. However, for small species with a short lifespan, the environment-dependent demographic data from the field are usually not available. Their collection is either not possible at all or not economically practical. Thus, estimating effects of environmen-

tal stochasticity on small short-lived organisms needs a different approach, which takes these issues into consideration. In this study, we combined laboratory-based demographic data and actual climatic data to estimate these parameters; which is detailed in Chapter 3 of the Thesis.

4.2.6 Estimating the influence of density dependence

The common practice to include a density-dependent function in population matrix models is to use Beverton-Holt or Ricker type models [26, 27, 37, 38]. Morris and Doak [38] suggested that the following important questions should be taken into account when formulating density dependent matrix models;

- which vital rates are density dependent?
- how do those rates change with density?
- which classes contribute to the density that each vital rate "feels"?

The issue of population density of *F. candida* has been previously discussed in a number of articles. Although different specific hypotheses have been put forward by different authors, they all concluded that higher population density indeed influences the vital rates of *F. can-*

didida. For example, Green [39] found that if the area per individual drops below 1.2 cm^2 the fecundity is reduced as a function of juvenile and adult population density. He suggested that a physiological effect, caused by a high population density, prevents the full development of the reproductive organs of *F. candida*, which depresses individual fecundity. He further stated that the reduction in fecundity is a manifestation of “stress” caused by jostling. On the other hand, Usher et al. [40] showed that although stress caused by overcrowding limits the population growth rate, the availability of food is the most important factor both in regulating the rate of population growth and in determining the maximum population density of *F. candida*. However, in their experiment, they used a population density well above the estimated collembolan population densities in the field. Despite the maximum 10 individuals per cm^2 carrying capacity reported by Petersen and Luxton [41]; Usher et al. [40] conducted their experiment at population densities ranging from 20 to over 60 individuals per cm^2 . Noel [42] found that crowding in *F. candida* populations results in reduced reproductive output and reduced somatic growth rate which, in turn, reduce population growth rate. Because the population densities used by Green [39] and Noel [42] were comparable to those reported by Petersen and Luxton [41] in the field, we considered the density-dependent mechanisms suggested by these

two studies. Furthermore, we assumed that the reproductive rate of all ages is influenced by population density to the same extent.

The reproductive rate of *F. candida*, F_3 , (which is already corrected for concentration x and temperature at time t) was, therefore, corrected further as a function of population density N using a Ricker type model as in Equation 4.9:

$$F_3(x, t, N) = F_3(x, t) e^{-\beta \left(\frac{N(x, t)}{A} \right)} \quad (4.9)$$

where

- $F_3(x, t, N)$: the number of eggs produced per individual per week at toxicant concentration x , temperature at time t and population size N ;
- $F_3(x, t)$: the number of eggs produced per individual per week at toxicant concentration x , temperature at time t and population density ≤ 1 individual per cm^2
- $N(x, t)$: total population size of juveniles and adults
- A : area of the study site (the unit depends on the scale of the study site)

- β : a constant that measures the strength of density dependence (area per individual).

Substituting Equation 4.5 and 4.9 into 4.2 and taking into consideration the temporal variation of the vital rates due to temperature, we get the stochastic stage-based density-dependent matrix model of Equation 4.10

$$\begin{bmatrix} N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} = \begin{bmatrix} P_1(x,t) & 0 & F_3(x,t) e^{-\beta \left(\frac{N(x,t)}{A} \right)} \\ G_1(x,t) & P_2(x,t) & 0 \\ 0 & G_2(x,t) & P_3(x,t) \end{bmatrix} \times \begin{bmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \end{bmatrix} \quad (4.10)$$

4.2.7 Mean Time to Extinction (MTE) as a measure of population-level effects

In this study we used the mean extinction time (MET) and the reduction of mean extinction time (ΔMET) as a population-level endpoint for assessing effects of chemicals. Although the method is not new for conservation biology and ecotoxicology, there are different approaches for estimating MET. Tanka and Nakanishi [43] performed a numerical comparison on the analytical and simulation techniques suggested by Hakoyama and Iwasa [44], Foley [45] and Landa [46] for evaluating the MET. Despite the differences in absolute values of expected METs,

they found fairly consistent tendencies among the three methods. In this study, we used the analytical technique provided by Lande [46]. As suggested by Lande [46], starting from a given initial population size, N_0 , the mean time to extinction, denoted as $T = T(N_0)$, is the solution of

$$\frac{1}{2}\sigma^2(N_0)\frac{d^2T}{dN_0^2} + \mu(N_0)\frac{dT}{dN_0} = -1 \quad (4.11)$$

where; $\mu(N_0)$ and $\sigma^2(N_0)$ are the infinitesimal mean and variance of population density. Considering the boundary conditions $T(1) = 0$ and $\frac{dT}{dN_0}|_{N_0=K} = 0$, i.e. the rate of change of the mean extinction time T at carrying capacity K is zero, Lande [46] derived a general solution for Equation 4.11 of the following form:

$$T = 2 \int_1^{N_0} e^{-G(z)} \int_z^K \frac{e^{-G(y)}}{\sigma^2(y)} dy dz \quad (4.12a)$$

$$G(y) = \int_1^{N_0} \frac{\mu(N)}{\sigma^2(N)} dN \quad (4.12b)$$

At a given carrying capacity, K , mean intrinsic population growth rate (r) and environmental variance (V_e), Lande [46] also estimated the mean time to extinction (T) by considering only the effect of

environmental stochasticity as:

$$T = \frac{2}{V_e c} \left(\frac{K^c - 1}{c} - \ln K \right) \quad (4.13a)$$

$$c = \frac{2r}{V_e} - 1 \quad (4.13b)$$

Based on the value of the mean intrinsic population growth rate, r , the author [46] further simplified Equation 4.13 a for the following two conditions. If r is positive (i.e. exponentially growing population), the MET can be evaluated as

$$T \approx \frac{2K^c}{V_e c^2} \quad (4.14)$$

If r is negative (i.e. exponentially declining population), the MET is given by

$$T \approx \frac{-\log K - \frac{1}{c}}{r} \quad (4.15)$$

The percentage reduction of the mean extinction time (ΔMET) due to the effect of the chemical is expressed as:

$$\Delta MET = \frac{MET_{control} - MET_{exposed}}{MET_{control}} \times 100\% \quad (4.16)$$

where $MET_{control}$ and $MET_{exposed}$ represent the MET of control and exposed populations respectively. The analytical solution of the MET

and ΔMET calculations (i.e. equations 4.13, 4.14, 4.15 and 4.16) require values for environmental variance, V_e , arising from environmental stochasticity, equilibrium population size (K) and the intrinsic population growth rate (r) for concentration x . The estimation of $r(x)$ and $V_e(x)$ is straightforward. The temperature-dependent individual vital rates, corrected with the dose-response relationship (Equation 4.5), were used to construct the set of temperature-dependent projection matrices for each concentration level. This set of projection matrices, $\mathbf{A}(\mathbf{x}, t)$, and their environmental probability vectors $P(t)$ are substituted into Equation 4.6, 4.7 a and 4.7 b to determine $r(x)$ and $V_e(x)$.

The functional relationship between the carrying capacity K , and concentration x can be derived by re-writing equation 4.11 for the equilibrium population density case and applying the rules of matrix algebra. At the stable age distribution, the population projection matrix for the three-stage model of Equation 4.2 can be expressed in compact matrix form as follows:

$$[\mathbf{B}][X] = \lambda[X] \quad (4.17a)$$

$$([\mathbf{B}] - \lambda[\mathbf{I}])[X] = 0 \quad (4.17b)$$

where \mathbf{B} is the projection matrix of equation 4.2 , X is the right eigenvector of matrix \mathbf{B} , λ is the dominant eigenvalue of matrix \mathbf{B} and \mathbf{I} are a 3 by 3 identity matrices. For density dependent populations, λ converges to unity and X converges to equilibrium population density. Thus, equation 4.11 can be represented as:

$$\begin{bmatrix} P_1(x,t) - 1 & 0 & F_3(x,t) e^{-\beta \left(\frac{N(x,t)}{A} \right)} \\ G_1(x,t) & P_2(x,t) - 1 & 0 \\ 0 & G_2(x,t) & P_3(x,t) - 1 \end{bmatrix} \times \begin{bmatrix} X_1 \\ X_2 \\ X_3 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix} \quad (4.18)$$

From this argument, we can derive the characteristic equation of the coefficient matrix that can be expressed in a nonlinear Equation 4.19 (omitting x and t)

$$P_1 P_2 P_3 - P_1 P_2 - P_3 (P_1 + P_2) + (P_1 + P_2) + P_3 + G_1 G_2 F_0 e^{-\beta \frac{N_{eq}(x,t)}{A}} = 1 \quad (4.19)$$

Considering a unit study area and solving Equation 4.19 for $N_{eq}(x,t)$, the equilibrium population density for any chemical exposure level x , $N_{eq}(x,t)$, can be expressed mathematically as

$$N_{eq} = \frac{-1}{\beta} \ln \left(\frac{1}{G_1 G_2 F_3} (P_1 P_2 + P_1 P_3 - P_1 P_2 P_3 - (P_1 + P_2 + P_3)) \right) \quad (4.20)$$

Hayashi et al. [24] applied the same mathematical approach for the analysis of the population-level effect of Zn on the fathead minnow (*Pimephales promelas*).

Thus, correcting the vital rates using the dose-response relationship of Equation 4.5 and substituting into Equation 4.20 provide the equilibrium population size for any concentration level x , which eventually gives the relationships between Cd concentration and equilibrium population size of *F. candida*. All calculations and numerical simulations of the model were performed using MATLAB programming platform version 8.0.0.783 (R2012b).

4.3 Application to experimental data

The aforementioned modelling approach is demonstrated by predicting the population-level effect of Cd on *F. candida* populations living in the natural environment. We used data from Menta et al. [47] and Crommentuijn et al. [48] to fit and parameterize the dose-response relationship of Equation 4.5. Menta et al. [47] reported the effect of Cd on survival of eggs, survival of juveniles, and egg production at different concentration levels. Similarly, Crommentuijn et al. [48] reported the effect of Cd on survival of adults, development rate of eggs, development rate of juveniles, and population increase at different concentrations. We used the free plot reader software Engauge Dig-

itizer Version 4.1 (<http://digitizer.sourceforge.net>) to extract data from the dose-response graphs reported in these studies. The data on temperature-dependent vital rates obtained from previous studies were used to define important temperature regimes and parameterize the temperature-dependent matrices, $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_N$ in Equation 4.8. In addition to these secondary data sets, we conducted an eight-week life-history study at 15, 20 and 25 °C and observed egg development, juvenile development and reproductive rate as a function of temperature. These laboratory-based temperature-dependent demographic data combined with actual field soil temperatures were used to derive temperature-dependent matrixes and the environmental probability matrix P_i of Equation 4.6d. For this study, five years of daily temperature records (from 2006 to 2010) obtained from the Gaik-Brzezowa Research Station (Wieliczka Foothills) of the Institute of Geography and Spatial Management, Jagiellonian University, were used to derive an environmental probability matrix, P_i of Equation 4.6d. We fitted the empirical data set reported by Green [39] into the density-dependent function of Equation 4.7 to parameterize $F_3(x, t)$ and β using the MATLAB Curve fitting Toolbox.

In this analysis, we assumed that effects of Cd are independent of temperature and density-dependence. The calculation of λ is also made by assuming the population is under their demographic carrying ca-

capacity. For the case, the population are at their carrying capacity, we assumed the population is fluctuating around the equilibrium population size; and λ is unity.

4.4 Results

4.4.1 Effects of Cd at the individual level: Dose-Response Relationship

Figure 4.1 a-e show the dose-response relationships of the vital rates of *F. candida*, and Table 4.1 gives the values of the parameters of the dose-response (Equation 4.5) relationship for each vital rate affected by Cd estimated at the experimental temperature 20 °C.

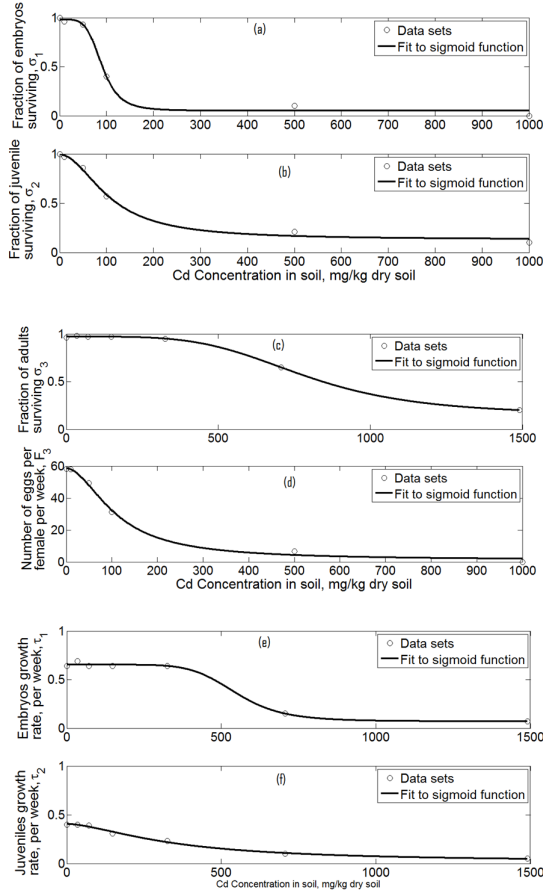


Figure 4.1: Dose-response relationships for the vital rates of *F. candida*. Data from ([47] and [48]). Embryo growth rate is expressed as the projection time (1 week) divided by the number of weeks an egg requires to hatch. Juvenile growth rate is expressed as the projection time (1 week) divided by the number of weeks a newly hatched juvenile requires to reach puberty.

Table 4.1: Parameter estimates of the dose-response relationship of Equation 4.5. D corresponds to the mean response of the control, C is the mean response at very high doses b describes the slope of the curve around the EC_{50} .

	Parameters			
Vital rates	C	D	EC_{50}	b
Egg survival, σ_1	0.05	0.98	90.1	4.8
Juvenile survival, σ_2	0.13	0.99	107	2
Adult survival, σ_3	0.15	0.99	788	4.2
Egg development, γ_1	0.07	0.65	550	7.3
Juvenile development, γ_2	0.01	0.41	343.6	1.6
Egg production, F_3	1.1	58.6	110	1.9

4.4.2 Temperature dependence of vital rates and estimating λ_s and σ_e^2

Based on the functional relationships between vital rates and temperature as well as the temperature conditions of the study area, eight temperature regimes that influence the vital rates of *F. candida* in a distinct way were identified (in °C): < 0 , 0-5, 6-8, 9-11, 12-15, 16-18, 19-22, and 23-25. The eight temperature regimes were used to derive and parameterize eight distinct projection matrices of 4.2 and to derive the environmental probability matrix $P_{i,j}$ for the eight temperature ranges. The eight temperature-dependent matrices constructed using the temperature-dependent vital rates together with their probability of occurrence, P_i , were used to estimate the stochas-

tic population growth rate, λ_s , and σ^2 using Equations 4.6, 4.7a and 4.7b.

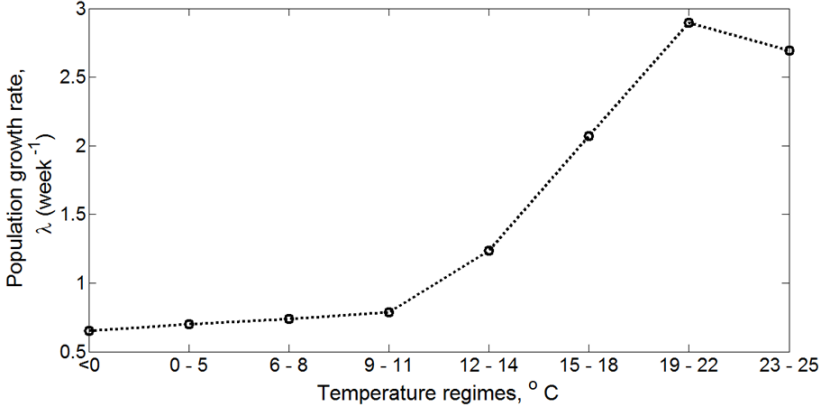


Figure 4.2: Population growth rate, λ , (per week) for the eight temperature regimes. Calculated based on the temperature dependent life history data obtained from unpublished and published data sets.

4.4.3 Effect on intrinsic population growth rate

The effect of Cd on the intrinsic population growth rate of *F. candida* was compared for three different cases as shown in Figure 4.3: two deterministic models (at a constant optimum temperature of 20 °C and at a constant mean weekly temperature for the study area of 11.7 °C and the stochastic population growth rate estimated using Equation 4.7 and considering weekly temperature fluctuations as described above. The optimum temperature produced the highest intrinsic growth rates across the whole range of tested Cd concentra-

tions while stochastic temperatures resulted in the lowest population growth rates. Furthermore, populations under stochasticity began declining ($\lambda < 1$) at a Cd concentration seven times lower than that for populations at the optimum temperature (100 vs 700 mg/kg, respectively; Figure 4.3).

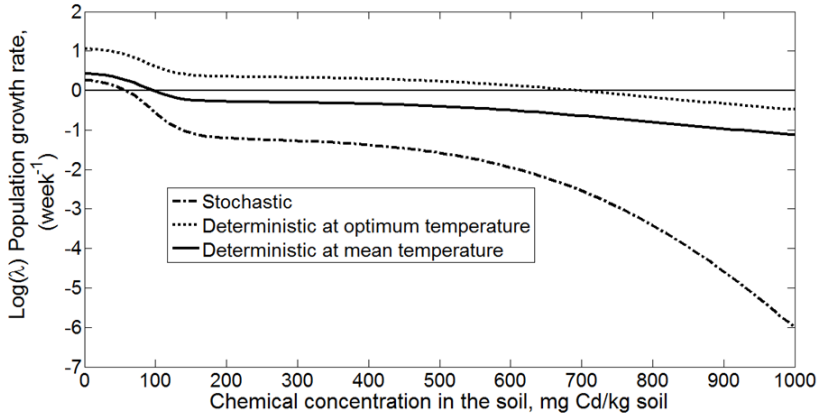


Figure 4.3: The intrinsic population growth rate λ (per week) for three different conditions (Deterministic at 20 °C:···, Deterministic at mean temperature:—, Stochastic: - -). The horizontal line represents the critical population growth rate, $r = 0$.

4.4.4 Effect on equilibrium population size

Assuming a study area of 1 square meter and the population at its carrying capacity, we estimated population density as a function of Cd concentration (Figure 4.5). Without Cd exposure, the equilibrium population size was estimated at around 79500 individuals per

sq.m, and with increasing Cd concentration it declined monotonically, reaching less than 8000 at 1000 mg Cd/kg. A particularly sharp decrease can be noted between 50–150 mg Cd/kg.

The decrease in the equilibrium population density might enhance the individual reproduction rate of *F. candida* (from Equation 4.9). Therefore, we calculated the individual reproduction rate at a range of Cd concentrations (Figure 4.5) using Equation 4.9. The reproduction rate of *F. candida* was estimated to be 9 eggs per female per week when they live at their carrying capacity in a clean environment (which is much lower than that of the density independent condition). Under Cd exposure, reproduction is not affected until ca. 30 mg/kg, and then it decreases slightly up to ca. 100 mg/kg. However, between 100 and 200 mg/kg the reproduction rate increased because of the decreased equilibrium population density, which results in partial compensation of toxic Cd effects. Above ca. 270 mg/kg of Cd, this partial compensation effect vanishes and the reproduction rate decreases monotonically with further increase in Cd concentration.

4.4.5 MTE & Δ MTE

As mentioned above, we used MTE as a risk assessment endpoint for the combined effect of Cd, seasonal environmental variability, and density dependence. Figure 4.6 shows MTEs calculated at different

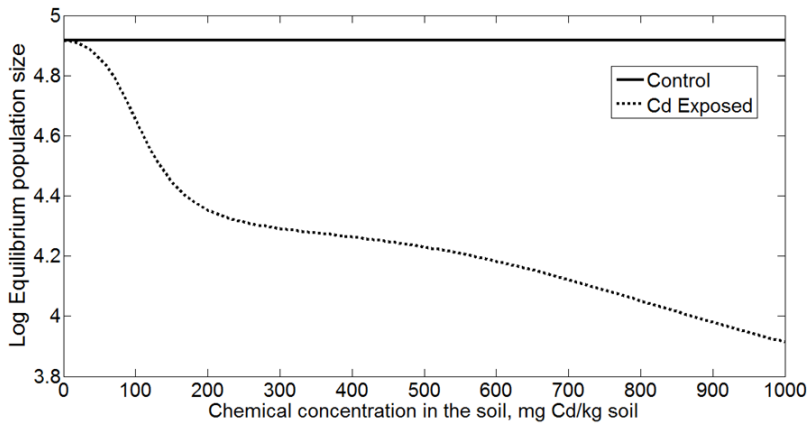


Figure 4.4: The decrease in equilibrium population size of *F. candida* with increasing cadmium concentration in soil.

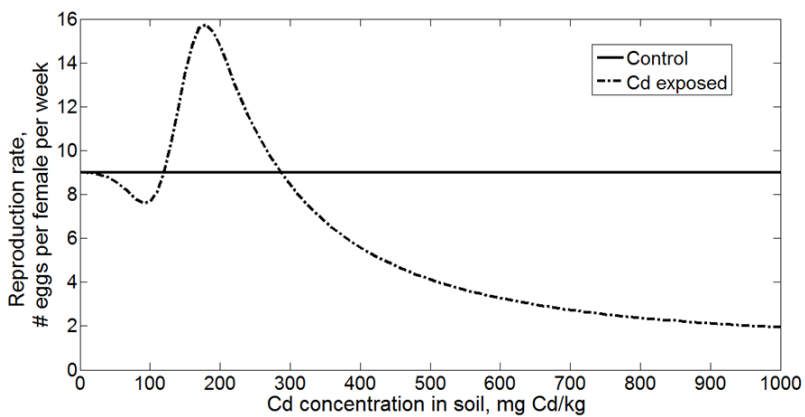


Figure 4.5: The reproduction rate of *F. candida* as a function of Cd concentration in the soil.

Cd concentrations, and Figure 4.7 shows the reduction of the MTE as a function of Cd concentration in the soil. A sharp decrease of

the MTE from $10^{5.46}$ weeks to $10^{5.32}$ weeks between the control and 300 mg/kg Cd was followed by a more gradual decrease to $10^{5.25}$ at 1000 mg/kg. This trend is the result of the combination of the sharp decrease of population growth rate, λ , for the Cd concentration between 0 and 200 mg/kg and the sharp decrease of the equilibrium population density between 50 and 150 mg/kg. Consequently, the percentage reduction of the MTE (Δ MTE) sharply increased from 0 to 25.3% between 0 and 300 mg/kg and gradually reached 38.5% at 1000 mg/kg.

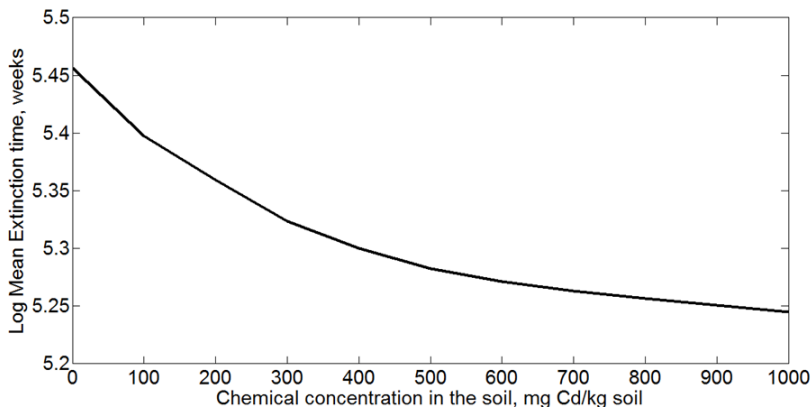


Figure 4.6: Mean extinction time, in weeks (log scale), as a function of Cd concentration soil. The mean extinction time (MET) for the control population is indicated by the solid straight line.

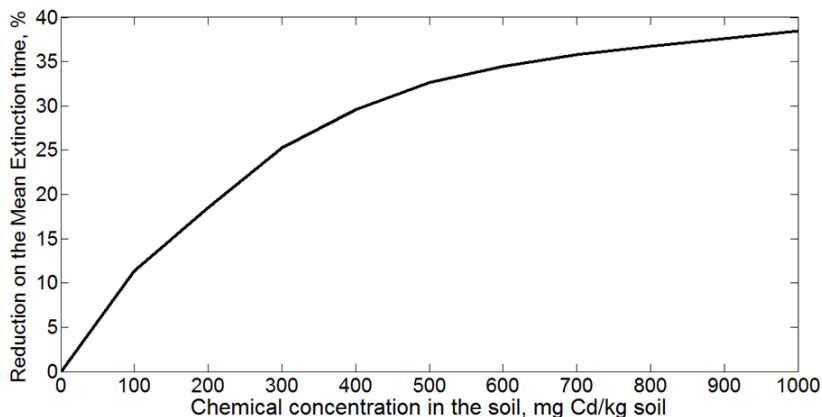


Figure 4.7: Reduction in the mean extinction time (expressed in % control) as a function of Cd concentration in the soil

4.5 Discussion

4.5.1 Model credibility

The extrapolation of individual-level effects observed in laboratory bioassays to population-level risks is one of the major challenges in ecotoxicology and ecological risk assessment of chemicals. The lack of ecological realism when measuring effects of chemicals at the individual level and the assumption that these effects translate directly to the population level are the main sources of controversy. Risks of chemicals at the population level can be strongly affected by stochastic events that can arise from population structure, density dependence, timing of exposure and environmental fluctuations that are not considered in laboratory experiments [10]. In this work we presented a

general framework to extrapolate individual-level effects to the population level using a stochastic matrix model which takes into account two important ecological factors: temporal temperature fluctuations and density-dependence. The proposed matrix model is a widely used and simple modelling framework for exploring the dynamics of populations, and it can be easily adapted for other similar problems. The conceptual framework of the model is derived from realistic ecological assumptions. The well-proven and tractable mathematical formulations used for the functional representation of the model make it robust and mechanistic.

The credibility of the model results can be evaluated by comparing them with results from published actual population-level studies. Gregoire–Wibo and Snider [50, 51] studied the full life cycle of *F. candida* at 15, 21 and 26.6 °C. They used these data to estimate the intrinsic population growth rates using the Euler-Lotka method and found that r was 0.089, 0.15 and 0.135 day⁻¹ at 15, 21 and 26.6 °C, respectively. These values are comparable to our model predictions for the respective temperature ranges, i.e., 15-18 °C (0.104 day⁻¹), 19-22 °C (0.152 day⁻¹) and 23-25 °C (0.142 day⁻¹). Similarly, Subaja [52] studied the full life history of *F. candida* at 15.5 °C and found that the intrinsic population growth was 0.09 day⁻¹, which is comparable to 0.104 day⁻¹ that we estimated for the temperature range of

15-18 °C. There are only limited studies available that have assessed population-level effects on *F. candida* exposed to Cd contaminated soil. Crommentuijn et al. [48] calculated the EC_{50} for population growth rate of *F. candida* at 470 mg/kg, whereas our model estimate is 520 mg/kg. Although somewhat higher, the value estimated by our model is well within the confidence interval (411-549 mg/kg) reported by Crommentuijn et al. [48]. In another study, Herbert et al. [53], using a dose-response relationship, estimated the Cd concentration at which the population growth rate would be zero (i.e., the critical concentration at which exponential growth reversed to exponential decline). This critical Cd concentration was 398 mg/kg, which is much lower than our prediction for the optimum temperature (690 mg/kg, as shown in Figure 4.3). This substantial difference may originate from using different measures of population growth: Herbert et al. [53] used the instantaneous population growth rate, while our model was formulated to estimate the intrinsic population growth rate. As shown by Walthall and Stark [54], these two measures do not always correlate closely. This is partly confirmed by a large difference between the instantaneous population growth rate calculated by Herbert et al. [53] for control population (0.05 day⁻¹) and population growth rates reported in other studies for the same temperature [48-51].

The general structure of the model is generic and can be easily adopted to any population-level assessment issue, for any species influenced by environmental fluctuations and in any environment (e.g. terrestrial or aquatic) and/or spatial location. The model can also be modified for any kind of density dependence. Due consideration has been also given to the data requirements to adopt and use the model. As illustrated in our case study, the model can be implemented with easily available and/or measurable data. Most of the individual-level toxicity data for *F. candida* can be obtained either from published literature or from the OECD guideline standard toxicity tests. For new chemicals, these individual-level effects can be easily acquired from partial and full life-history studies. But it is worth mentioning that the standard soil toxicity alone has some limitations to provide all the endpoints required for the three-stage model we developed here. For example, according to ISO and OECD guidelines the reproduction of *F. candida* is determined by counting the number of juveniles at the end of the experiment (after 28 days). But this endpoint is the combination of the reproduction rate, survival of embryos and survival of early juveniles. It is also difficult to count eggs from soil since these organisms lay eggs inside the soil. Although counting eggs in the soil might have some technical difficulties, it is possible to minimize the uncertainties by following the number of juveniles at

different time periods. Thus, including this provision in the standard toxicity tests facilitates the direct use of the information in models like this. It is also possible to use the standard toxicity test results by modifying our model to a two-stage model, i.e. without considering the embryonic stage. However, predictions from the two stages based model is not only prone to higher uncertainties [26,38] but also effect of environmental factors on the embryonic stage will be overlooked.

4.5.2 Extrapolating individual-level effects to the population level

As discussed by a number of authors (e.g., [2,3,37]), the extrapolation of individual-level effects to higher levels of biological organization, (e.g., to the population level) without considering environmental variability may underestimate toxicant effects. On the basis of this assumption, we compared the population growth rate, one of the most important population-level endpoints [17,55], under three temperature regimes. The comparison showed that the direct translation of individual-level ecotoxicity tests without considering seasonal temperature variations significantly overestimates population growth rate. As shown in Figure 4.2, the population growth rate estimated for the temperature range of 19–22 °C (the standard temperature for ecotoxicity tests with collembolans) is much higher than all other

population growth rates estimated at different temperature regimes. It should also be remembered that the mean soil temperature in most of Europe is much lower than the 20 °C used in standard ecotoxicity tests. Our findings clearly showed that environmental stochasticity influences the effect of Cd on *F. candida* populations. As shown in Figure 4.3, the stochastic population growth rate is significantly lower than the deterministic growth rate (i.e., $\log\lambda_1$ and $\log\lambda_s$ in equations 4.6, respectively) at higher Cd concentrations (Figure 4.2). This is because of the increased temporal variance and co-variance among the vital rates. The simulation results reveal that a model based only on average vital rates may overestimate growth rates of populations living in natural environments which experience variable environmental conditions.

4.5.3 Population-level effects of chemicals for density–dependent conditions

Density dependence is a fundamental concept in population biology, and it is clear that density dependence in some form is exhibited by most species [2]. Population models that do not include this important ecological factor might result in either false positive or false negative population-level effects of chemicals. Density dependence should therefore be an integral part of ecotoxicology and ecological

risk assessments [2]. Theoretical and empirical studies have demonstrated that toxicant effects at the population level may be modified by density-dependent processes. One potential consequence of chemical exposure of density-limited populations is that, by removing a fraction of individuals, the chemical reduces the intensity of density dependence and thus has less of an impact on population dynamics than if the population had been in exponential growth [4]. Indeed, our model shows that the negative effect of Cd on population density resulted in compensatory effects on the reproduction rate, the vital rate affected by density regulation in *F. candida*, between 100-180 mg/kg soil concentration (Figure 4.5). However, above ca. 180 mg/kg the compensatory effect disappeared, showing that partial compensation of toxic effects by density-dependent mechanisms is limited to a relatively narrow range of toxicant concentration. From a population dynamics point of view it is thus difficult to interpret this partial compensation.

4.5.4 MTE as an endpoint to assess ecological risk of chemicals

Different endpoints have been proposed to assess and interpret the population-level effects of chemical. For instance, intrinsic population growth rate [17,55], or the equilibrium population size [22,24,37],

which assumes that the population in the environment always exists at carrying capacity . However, the dynamics of an animal population living in a natural temporally variable environment cannot be represented with exponential growth or constant density at population carrying capacity; but rather is expected to change as a function of the state of the environment. This is particularly important for short-lived ectothermic species whose vital rates are highly influenced by seasonal temperature fluctuations. For example, during the winter time, the population sizes of arthropods like *F. candida* are at their lowest levels. When the winter is over and the temperature is suitable for reproduction, they start growing exponentially at the rate equivalent to their natural growth rate before reaching a constant population density, and stay at this level with some fluctuation due to stochasticity until the temperature drops again. If such a population is additionally exposed to a toxic chemical, the population either goes to extinction or manages to persist, depending on the degree of the individual-level effects of the chemical and the current population dynamics resulting from natural environmental factors. If we consider the latter case (population persistence), the population will follow a trend similar to the unexposed population but with new parameters. Like the unexposed population, the population would drop during the winter to its lowest density, but this density is expected to be lower

than in an unexposed population. If the concentration of the chemical is not too high, the population will start to grow exponentially during the growing season but the growth rate would be lower than in the unexposed population. Though it might take a longer time, ultimately the population will reach a new equilibrium size, which again is expected to be lower than in the case of an unexposed population in the same environmental conditions. If the pollution is persistent, the population will permanently remain at lower densities which, in turn, increases the extinction probability due to environmental and demographic stochasticity. The endpoint that can combine all these factors into a common unit is MTE. Therefore, we argue that MTE and reduction of mean time to extinction (Δ MTE) are suitable population-level endpoints for assessing the risk of chemicals for populations in the natural environment. As shown in Figure 4.7, the MTE of *F. candida* decreases monotonically as a function of Cd concentration. This trend follows that of the equilibrium population size (Figure 4.4) and the population growth rate (Figure 4.3), which is expected from the mathematical relationships between the three indexes. From an ecological risk assessment perspective, the reduction in the MTE (Δ MTE) is more informative. As shown in Figure 4.7, we can easily estimate the percentage reduction of the MTE at different Cd concentrations. This endpoint is a convenient descriptor of what would

happen to the Cd exposed population compared to the control population and provides a quantitative measure in decision making process. However, MTE and Δ MTE could be more informative endpoint if we have preset criteria to decide what MTE or what percentage decrease in MTE is unacceptable. For example, as shown in Figure 4.7, despite the fact that the effect of Cd at its highest concentration seems large (almost 40% reduction of the mean time to extinction), even in this extreme case the estimated time to extinction is over 3000 years. We cross-checked our simulation results using a general protocol described in Grimm and Wissel [56]. The protocol provides a general mathematical structure to evaluate stochastic simulation models of population dynamics and quantifies important parameters in population viability analysis, including MTE [56]. Thus, using the protocol, we estimated the MTE as function Cd concentration and compared the results with our findings (i.e., Figure 4.7) As shown in the Appendix part of this chapter of the thesis, the MET estimated using the protocol suggested by Grimm and Wissel [56] is lower than the MET values we obtained (see Figure 4.10 of the Appendix). However, in both cases the MET is very high; thus, the population may be considered not really endangered even by high Cd concentrations. On the other hand, from an ecosystem-level perspective, a large decrease in population size, even without its extinction, can cause significant neg-

ative effects on ecosystem functions; especially if a keystone species is affected by a toxicant. Although it is not possible at the moment to interpret population-level effects at the ecosystem scale, one possible improvement in risk assessment, and an extension of currently proposed methodology, would be to add yet another end-point which can be calculated using the approach presented herein, namely the equilibrium size of a population under pollution pressure.

4.6 Conclusions

The stochastic density-dependent model presented in this article may be a convenient tool for extrapolating bioassay data on toxicity of chemicals to population-level effects under more realistic field conditions. It requires only a few additional pieces of information on species biology, which can be gathered relatively easily in laboratory studies. With adequate knowledge on species biology, the model can be easily adapted to virtually any species and environmental situation, and can make use of data on actual environmental conditions thus, it can be used for site-specific ecological risk assessment. The model for *Folsomia candida* was parameterized using published data and our own experimental data on individual vital rates, and its credibility was tested against actual published data for population dynamics of the species. The model was able to estimate population-level rates

with good precision for a range of tested temperatures. We therefore believe that this modeling approach can be useful for ecological risk assessment in complex and dynamic environmental situations. It would be interesting to further validate the practical applicability of the model by verifying the model simulation results against actual studies of effects of Cd and other metals at different temperature regimes and at different laboratory population sizes.

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Appendix

Cross-checking the estimation of MTE and evaluating simulation results

In this study we used the mean time to extinction (MTE) and the reduction of mean time to extinction (Δ MTE) as a population-level endpoint for assessing effects of chemicals on population. We implemented the mathematical formulation suggested by Lande [46] to estimate the MTE and the method is applied to estimate MTE and Δ MTE for *Folsomia candida* population exposed to cadmium. In the aim of cross-checking our approach and results, we applied a general protocol described by Grimm and Wissel [56]. The protocol provides a general mathematical structure to evaluate stochastic simulation models of population dynamics. The technique presented in the protocol is also quantifies important parameters in population viability analysis, including MTE [56]. Thus, using the protocol we estimated the MTE as function Cd concentration and compared the results with our findings (i.e., Figure 4.6) and revised our conclusion accordingly.

Brief description of the protocol

As described by Grimm and Wissel [56], the implementation of the protocol is based on two steps: first the estimation of the cumulative probability of extinction with time t , $P_0(t)$, and then transforming

the $P_0(t)$ data and fitting a linear function of Equation 4.21

$$-\ln(1 - P_0(t)) = \frac{1}{MTE}t + C \quad (4.21)$$

The linearity of the $-\ln(1 - P_0(t))$ vs. t plot, which is referred as the " $\ln(1 - P_0(t))$ - plot" in [56], helps to test the adequacy of methods for estimating $P_0(t)$; while the slope of the plot provides estimates on MTE (see [56] for detailed description of the protocol and mathematical details).

Implementation of the protocol

As discussed above, to implement the protocol we need to determine the cumulative probability of extinction with time $P_0(t)$. We applied the diffusion approximations of Equation 4.22 and Equation 4.23 (as suggested by Lande and Orzack [57]) to calculate the probability of extinction, $P_i(t)$ in interval t to $t + dt$ and cumulative probability, $P_0(t)$, that the population becomes extinct before time t .

$$P_i(t) = \frac{d}{\sqrt{2\pi\sigma^2t^3}} e^{\left(\frac{(-d + \mu t)^2}{2\sigma^2t}\right)} \quad (4.22)$$

$$P_0(t) = \phi\left[\frac{-d - \mu t}{\sqrt{\sigma^2t}}\right] + e^{\left(\frac{-2\mu d}{\sigma^2}\right)} \phi\left[1 - \frac{-d - \mu t}{\sqrt{\sigma^2t}}\right] \quad (4.23)$$

Where μ is the intrinsic population growth rate, σ^2 represents the temporal variation of the population growth rate, μ and d represents the difference between the log of current population density, N_c and the log of the threshold population density for extinction, N_x (i.e., $d = \log(N_c \log(N_x))$). $\phi[\zeta]$ is the cumulative distribution function for a standard Normal (mean = 0, variance = 1), and mathematical expresses as:

$$\phi[\zeta] = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\zeta} e^{-\frac{z^2}{2}} dz \quad (4.24)$$

To evaluate Equation 4.22 and 4.23 we had to substitute the values of three variables: μ , σ^2 and d . We assumed the population is existing at their equilibrium size (refer Figure 4.4 of the main paper); thus μ is zero. However, due to environmental variability μ fluctuates around the mean value. We used the σ^2 estimated using the Tuljapurkar's analytical method. The variable d can be estimated by setting a quasi-extinction threshold, N_x . We assumed $N_x = 1$, the population density at which there is total extinction.

Results

Figure 4.8 show the cumulative probability of extinction, P_0 as function of simulation time, t for different cadmium concentration. We fitted Equation 4.21 to the data after making transformation to form. Figure 4.9 depicts Equation 4.21 fitted to the transformed data. We

applied the MATLAB's built in function fit to estimate the coefficients of Equation 4.21. The MTE estimated from the $\ln(1 - P_0(t))$ - plot (together with the lower and upper 95 % CIs), along with the MTE reported in the main paper (i.e. Figure 4.6) are shown in Figure 4.10.

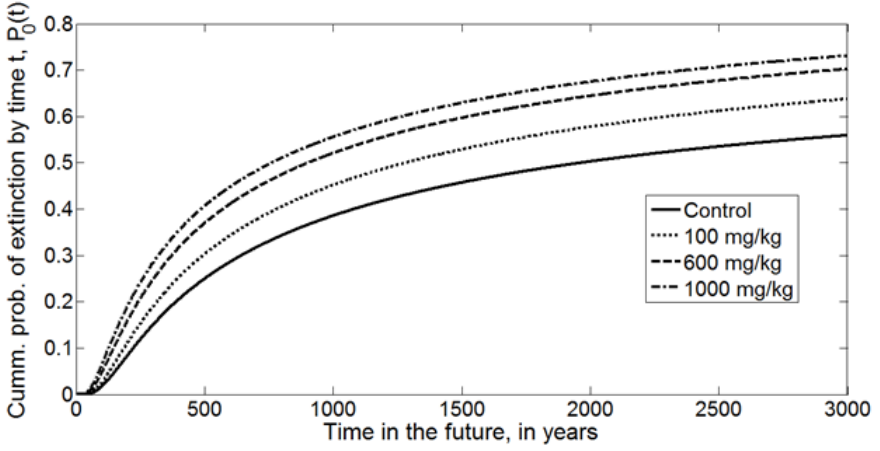


Figure 4.8: Cumulative probability of extinction with time t , $P_0(t)$, for selected Cd concentrations.

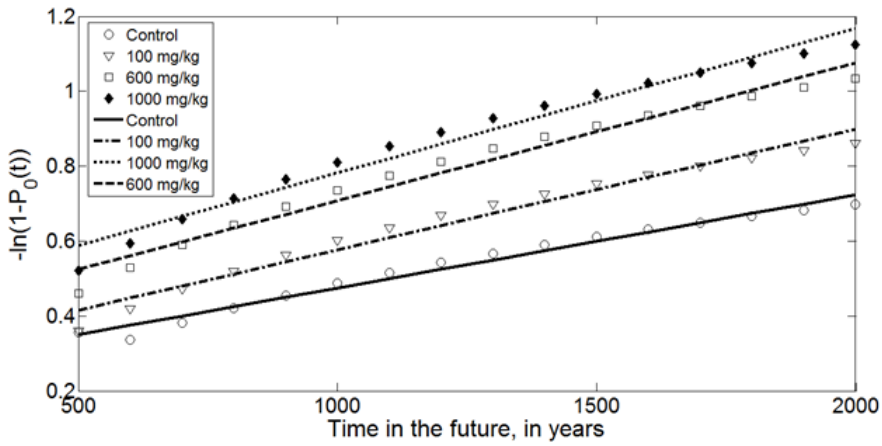


Figure 4.9: The $\ln(1 - P_0(t))$ – plot: $-\ln(1 - P_0(t))$ vs. t (points) and linear equations, with slope of years $\frac{1}{MTE}$, years⁻¹ (i.e., Equation 4.21) fitted to data points at different Cd concentrations (lines).

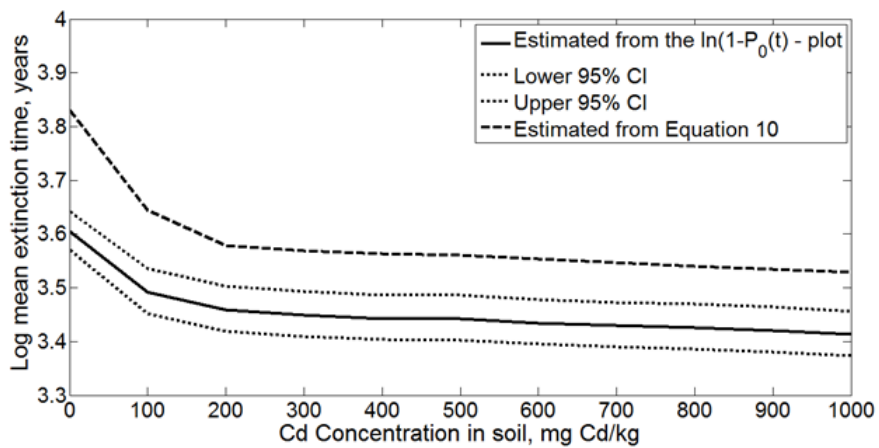


Figure 4.10: Mean time to extinction (MTE), in years (log scale), as a function of Cd concentration soil; estimated 1) from the $\ln(1 - P_0(t))$ – plot (solid line) together with the lower and upper 95 % CIs (small broken lines) and 2) using Equation 4.11 of the main paper (long broken lines).

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Chapter 5

Simplified DEB model to explore the effects of environmental stressors on the life history and population dynamics of *Folsomia candida*

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Laskowski R. Simplified energy-budget model to explore the effects
of environmental stressors on life history and population dynamics of
Folsomia candida

Abstract

Energetic models based on the dynamic energy budget (DEB) theory offer a mechanistic interpretation of how organisms acquire and use energy from the environment; they also serve as analytical tools to understand how fitness of organisms influenced by environmental stressors. In this study, we use a simplified energy-budget model (DEBkiss) to explore the effects of food limitation and temperature on the life history and population growth rate of the springtail *Folsomia candida*. We show how the various model parameters can be obtained by performing simple life-cycle experiments and observing standard end-points at different environmental conditions. At the early juvenile stage, the growth of *F. candida* deviated from the model predictions. We explain this as initial food limitation and suggest a model extension. Using the Arrhenius relationship, and correcting the rate related parameters for the effect of temperature, we are able to describe the effect of temperature on reproduction and growth. We show that the life history of *F. candida* is influenced by both food limitation and temperature in a predictable manner, which is captured by the model. However, at 25 °C, the model over-predicted the cumulative reproduction rate, which likely relates to the fact that 25 °C is near the highest threshold temperature for reproduction.

Using our model, we are able to predict the combined effect of food

density and temperature on the population growth rate of the organism. We show that food limitation and temperature can have different interactions on the population growth of *F. candida*.

Key words: energy-budget model, DEB, DEBkiss, *Folsomia candida*, environmental stressors, organism – environment interactions

5.1 Introduction

The physiological processes at the individual level determine fitness of organisms and the population dynamics emerging at higher levels of biological organization. These individual-level processes are a function of the state of the organism and its environment. Understanding these functional relationships is critical for addressing a wide range of problems in the basic and applied sciences of biology and ecology. Particularly, in recent years, predicting the short and long-term effects of environmental changes, like climate change and environmental pollution, on viability of habitats has become the center of discussion. Although empirical studies might help to understand some of these interactions, they are not effective for addressing all possible functional relationships and interactions. It is both economically prohibitive and technically difficult to assess all possible organism–environment interactions experimentally, and it is also dangerous to extrapolate results beyond the range of the observation points.

Thus, generalized mechanistic models were advocated for understanding basic questions in biology. One such modelling approach is the use of energetic models [1, 2]. Analogues to models for the physical world, energetic models are based on conservation laws (i.e., organisms obey conservation of energy in acquiring and using resources to fuel their life cycle). Thus, these models are founded on simple

mechanistic descriptions of how individual organisms take up and use energy and materials [1]. Different types of energetic based models for organisms have been developed (see e.g., [1,2]), but Dynamic Energy Budget (DEB) theory developed by Kooijman [3] is a well established and widely applied theory [4–7]. DEB theory captures the processes of development, growth, maintenance, reproduction and ageing for any kind of organism throughout its life-cycle [3]. A coherent set of assumptions leads to the basic DEB animal model, which is supported by empirical facts [8]. These features make DEB a general and comprehensive framework for different model studies and practical applications.

In this study, we applied a simplified DEB model (DEBkiss, [9]) to explore the effect of food limitation and temperature on the life history and population growth of the springtail *Folsomia candida*. The model allows for a mechanistic interpretation of the effect of food limitation and temperature on individuals. Primarily, we focused on estimating the parameters involved in the model by performing experiments, specifically designed for this purpose. Experimental studies were conducted in three different feeding conditions and three temperature regimes. The parameterized model was subsequently applied to explore the effect of food availability and temperature on the population growth rate of the organism. To extrapolate effects to the

population growth, the Euler-Lotka equation was used. The combined effect of food limitation and temperature on the population growth rate is investigated using model simulations.

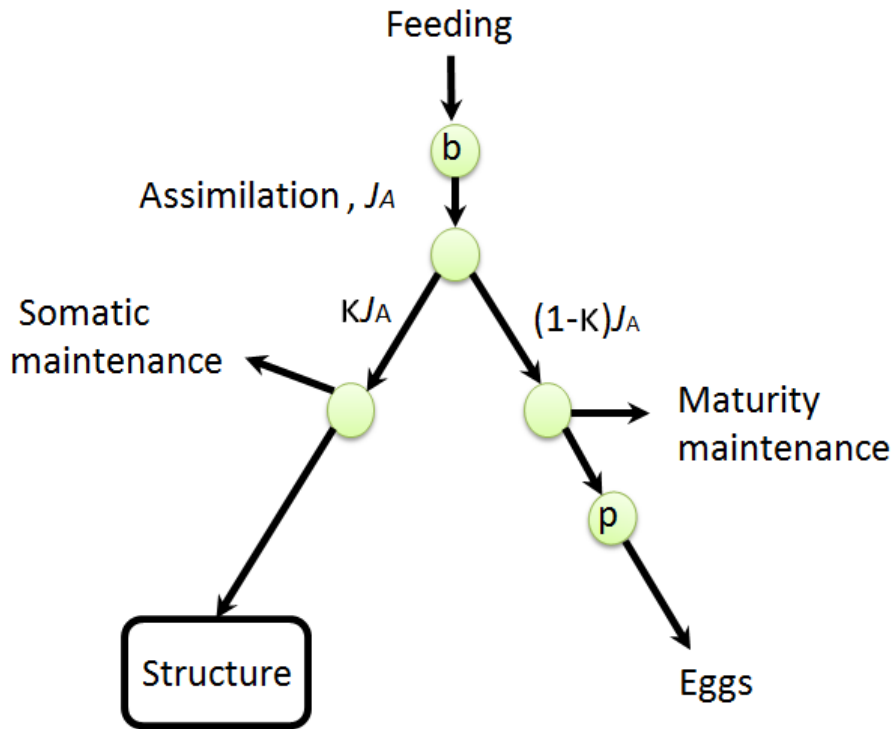


Figure 5.1: Schematic diagram of the DEBkiss model we applied in the study. The flow of mass indicated with arrows. The assimilated flux, J_A , is distributed in a fixed fraction (κ) between somatic growth/maintenance and maturity maintenance/reproduction. The circles containing “b” and “p” indicate maturity switches for birth and puberty, where feeding and allocation to reproduction begin, respectively.

5.2 Materials and methods

5.2.1 Experimental study

Study organism

A laboratory culture of *F. candida* was used for this experiment, which has been kept for more than 10 years at the Institute of Environmental Sciences of the Jagiellonian University, Poland. The animals were maintained as stock cultures in plastic boxes filled with moist plaster of paris, mixed with charcoal, at a constant temperature of 20 °C, and with dried baker's yeast (Dr. Oetker) as food. Prior to the experiments, adult animals were transferred to plastic boxes (10 cm in diameter) with plastic screw top lids, and filled with moist plaster of paris to lay eggs. After 7 days, the adults were removed and the eggs were allowed to hatch. Emerging juveniles of age between 1 and 3 days were used in the experiments.

Experimental design

Two sets of experiments were designed. In the first set, growth and reproduction of individuals was observed at three feeding conditions. In the second set, growth and reproduction of individuals was observed at three different temperatures with ad libitum food. Both experiments were carried out in plastic containers (3.5 cm in diameter) with plastic screw top lids. 1-3 days old juveniles were individually kept

in plastic vessels (diameter 3.5 cm) filled with moist plaster of paris. Baker's yeast solution (0.2 g/ml) was used as food source. To study the effects of food limitation, three food regimes were applied. In the highest food regime, food was supplied *ad libitum*. In the medium food regime, the yeast solution was available for 24 hours, followed by 48 hours without food. In the lowest food regime, the yeast solution was available for 24 hours, followed by 96 hours without food. For the temperature experiment, three constant temperature chambers set at 15, 20 and 25 °C were used. The humidity of the plaster of paris was kept constant by regularly spraying with distilled water. Equal number of 10 individuals per feeding and temperature regime were used. Body length and reproduction of the individuals were regularly determined for 11-12 weeks.

Digital image processing equipment was used to record individual body lengths (recorded every day for the first 3 weeks every 2–4 days until the seventh week, and once per week thereafter until the end of the experiment). Body lengths (i.e. the distance from the posterior end of the abdomen to the anterior end of the head between the antennae) were determined using the free image analysis software ImageJ (v.1.47p); (National Institute of Health, USA, <http://imagej.nih.gov>).

Individuals were monitored and recorded every day for egg produc-

tion using a microscope. When eggs were observed, the individuals were transferred (since this species is known to eat its eggs), and high-resolution photographs were taken. The eggs were counted using ImageJ. Beside these continuous observations, the diameter of the eggs at first reproduction and weight (i.e., dry and wet) of individuals at the end of the experiment were measured.

5.2.2 DEBkiss model

The DEBkiss model used in this study was developed and described in detail by Jager et al. [9]. The model describes and follows mass fluxes as dry weight. Figure 5.1 depicts the state variables and energy/material flows. The most important difference with the standard DEB animal model [8] is that DEBkiss does not include a reserve compartment (all body mass is treated as structure), and lacks a state variable for maturity (investment in reproduction starts at a fixed body size). Here, we included the process of maturity maintenance, which is not part of the simplest possible DEBkiss model [9].

The full DEBkiss model applies three state variables: egg buffer, structural body mass, and reproduction buffer, and involves seven mass fluxes: feeding, assimilation, structural growth, the flux into the reproduction buffer, maturity maintenance, and somatic maintenance. For the purpose of this study, we ignored the details of the

feeding process. Furthermore, we do not focus on embryonic development, and fit the model using continuous reproduction. This means that the egg and reproduction buffer were not followed as state variables (a detailed mathematical description of the model can be found in the supplementary material).

5.2.3 Modelling the effect of environmental stressors

The effect of environmental stressors can be included in the model by altering one or more of the DEBkiss model parameters. In our case, we only considered food density and temperature as the environmental stressors. The effect of food availability can be included by altering the scaled functional response, f as function of food density. The scaled functional response is the actual ingestion rate of an individual, divided by the maximum ingestion rate for its size. Its value is therefore restricted between 1 (ad libitum food supply) and 0 (complete starvation). The effect of temperature is included by scaling all rate parameters by the same factor. Therefore, the maximum area-specific assimilation rate (J_{Am}^a) and the volume-specific maintenance costs (J_M^v) are corrected for temperature and are multiplied by the same factor. Parameter values estimated at 20 °C (293 K) were used as reference and corrected for any other temperature (T in Kelvin) multiplied by an Arrhenius correction factor (F_T), mathematically

expressed as:

$$F_T = e^{\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right)} \quad (5.1)$$

where T_A is the Arrhenius temperature constant, in Kelvin and T_{ref} is the reference temperature, in Kelvin.

5.2.4 Deviation of the growth model

The growth of *F. candida* deviates from the von Bertalanffy growth curve, as predicted from the DEBkiss model, at its early stage. Two different hypotheses have been suggested for such deviations. Kooijman et al. [10] and Augustine et al. [11] suggested an acceleration of metabolic performance for some time after birth. This hypothesis failed to explain the specific pattern of our data. Alternatively, Jager et al. [12] suggested a size-dependent food limitation at the early juvenile stage. The recent study by Zimmer et al. [13] clearly showed how initial food limitation influenced the growth of the pond snail *Lymnaea stagnalis*. Food limitation is included in their model by assuming that the scaled functional response f is a function of body size. In the latter approach, they assumed a critical structural body length above which the food limitation disappears. This constant critical structural body length, however, was not indicated for our data. As shown in Figure 5.2(a) (top) and 5.3(a) (top) all growth curves start

to follow a von Bertalanffy type curve at different body length for different food regimes, but roughly at the same age (after temperature correction). To account for this behavior, we assume a stress factor on feeding (s_f), for the early-juvenile period (i.e., $a < T_f$). Therefore, we multiply the scaled functional response by s_f (value of less than unity):

$$f = \begin{cases} s_f f^* & \text{if } a < T_f \\ f^* & \text{if } a \geq T_f \end{cases} \quad (5.2)$$

f^* indicates the food availability in the environment, whereas f is the actual food level that the organism experiences (which is less for early juveniles), a is the age (in day) of the animal after hatching, and T_f is the critical age above which juveniles efficiently feed at the maximum level. Thus, we introduced s_f and T_f as new model parameters to be estimated.

5.2.5 Parameter estimation

The data obtained from the feeding experiment were used to estimate all the biological parameters involved in the DEBkiss model, while the data from the temperature experiment were only used to estimate the Arrhenius temperature, T_A and to validate the parameter set. First, we estimated the shape correction coefficient δ_M and the dry weight density of structure d_V from the dry weight, wet weight and physical

body length measurements, which were taken at the end of the feeding experiment (See the Appendix part). The shape correction factor, δ_M was used to translate body length observations to body mass, which is needed to ensure mass balance in the model. The diameter of the egg is used to estimate the volume of the egg (by assuming the egg is a perfect sphere), and this volume is converted to dry weight to yield the egg weight W_{B0} . If we assume a negligible somatic maintenance cost for a newly hatched juvenile, the body mass at birth W_{Vb} can easily be calculated from egg weight (see Equation 5.14a in the Appendix). This body mass at birth is used as initial value for fitting the growth and reproduction data.

For ad libitum food conditions, we have to take $f = 1$, while for the two limiting food conditions, f is a parameter that needs to be estimated. For the yield coefficients in Table 5.1, defaults were used [9], as they cannot be independently estimated from the available data. The estimation of the other model parameters was performed by fitting the growth and reproduction data simultaneously in two steps. In the first step; the length and reproduction data from the three feeding conditions were used simultaneously to estimate all the biological parameters, the scaled functional response, for the two food limited conditions (i.e. f_2 and f_3) as well as early stage juvenile food limitation parameters, T_f and s_f , which we assumed to be independent

of food level. In the second step, the parameters estimated from the first step were employed to estimate the Arrhenius temperature, T_A and yield of egg buffer on assimilates, y_{BA} , at 25 °C. The model with fixed biological parameters is simultaneously fitted to the growth and reproduction data from the three temperature conditions to optimize the value of T_A . We assumed s_f is temperature independent, and we used the value estimated from the feeding experiment for all three temperatures. However, as T_f is a physiological time, its value needs to be corrected by dividing it with the Arrhenius correction factor. In both parameterization steps, we used maximum likelihood optimization (assuming independent normal distributions for the residuals after square root-transformation of the growth and reproduction data), and derived the confidence intervals by profiling the likelihood (detailed description of the method can be found in [14]). All calculations were performed with a program code developed in MATLAB (Version 8.0.0.783, R2012b).

5.2.6 Model simulations: predicting population-level effects

We linked the DEBkiss model with the Euler-Lotka population equation (Equation 5.3) to extrapolate the individual-level measurements and predictions to the population-level. We used this equation to predict the effects of food density and temperature on the intrinsic

population growth rate.

$$\int_{a_b}^{a_d} S(a)R(a)e^{-ra}da = 1 \quad (5.3)$$

Where a is age (in day, starting with the fresh egg at $a = 0$); $S(a)$ is the probability for an individual to survive up to age a ; $R(a)$ represents the reproduction rate of an individual of age a , (# eggs per day); r the intrinsic population growth rate, (day^{-1}). a_b is the age at birth (hatching time of eggs) and estimated using the relation described in Equation 5.14b of the Appendix; a_d is the time when we stopped the experiment after 85 days post hatching (the last date of observation for egg production).

We described the survival probability, $S(a)$ using a Weibull function. We assumed the survival of the embryos is 100%. We also assumed that the survival of *F. candida* is not affected by food density. However, the survival of *F. candida* is temperature dependent and at lower temperature they live longer [15]. Thus, we estimated the parameter for the Weibull function by fitting life history data reported by Snider and Butcher [24] at 15, 21 and 26 °C. We used the free plot reader software Engauge Digitizer Version 4.1 (<http://digitizer.sourceforge.net>) to extract data from the survival vs. age graph reported by the authors. Fits of the survival model

to data and the estimated value of the parameters are included in the Appendix part. Equation 5.3 is solved numerically for the intrinsic population growth rate, r using the bisection method. The iteration routine was implemented using a programme coded in Matlab 2012b. We further performed an analysis that aimed to characterize the nature of the interaction between food limitation and temperature. Depending on their nature of the interaction, the combined effect of the two stressors can be characterized as: 1) additive: if the combined effect of the two stressors is the same as the sum of their independent effect; 2) more than additive: if the combined effect is higher than the sum of their independent effect; and 3) less than additive: if the combined effect is less than the sum of their independent effect. For the analysis, we defined four population growth rates, $r_{T,f}$ (an optimum population growth rate, which is estimated at optimum temperature, T and ad libitum food condition, $f = 1$); $r_{T+\Delta T, f+\Delta f}$ (a population growth rate under sub-optimal temperature, $T + \Delta T$ and food density lower than ad libitum condition, $f + \Delta f$); $r_{T+\Delta T, f}$ (population growth rate at sub-optimal temperature, $T + \Delta T$ but ad libitum food condition, $f = 1$); and $r_{T, f+\Delta f}$ (population growth rate at optimal temperature but food limited, $f + \Delta f$). We define $\Delta r_{T+\Delta T, f+\Delta f}$ as the reduction of the population growth rate due to the combined effect of sub-optimal temperature and food limited con-

dition $(r_{T,f} - r_{T+\Delta T,f+\Delta f})$. Similarly, $\Delta r_{T+\Delta T,f}$ is the reduction of the population growth rate due to sub-optimal temperature condition alone $(r_{T,f} - r_{T+\Delta T,f})$; and $\Delta r_{T,f+\Delta f}$ is the reduction due to food limited condition alone (or $r_{T,f} - r_{T,f+\Delta f}$). Mathematically, the three interactions can be represented using Equation 5.4.

$$\Delta r_{T+\Delta T,f+\Delta f} \begin{cases} = \Delta r_{T+\Delta T,f} + \Delta r_{T,f+\Delta f} : \text{additive} \\ < \Delta r_{T+\Delta T,f} + \Delta r_{T,f+\Delta f} : \text{less than additive} \\ > \Delta r_{T+\Delta T,f} + \Delta r_{T,f+\Delta f} : \text{more than additive} \end{cases} \quad (5.4)$$

Quantitatively, we determined the nature of the interaction based on the sign of the difference between the two sides of Equation 5.4, as described in Equation 5.5.

$$\Delta r = \Delta r_{T+\Delta T,f+\Delta f} - (\Delta r_{T+\Delta T,f} + \Delta r_{T,f+\Delta f}) \quad (5.5)$$

Depending on the interaction, Δr in Equation 5.5 can be either negative, if the combined effect is less than additives, or positive, if the combined effect is more than additive, or zero, if the combined effect is additive.

5.3 Results

5.3.1 Parameter estimates, model fit and effect on growth and reproduction

Table 5.1 shows the estimated values for all parameters used in our model, while Figure 5.2 and Figure 5.3 show the model fit to the data. As shown in the figures, despite the relatively poor fit observed on the initial part of the growth curves, the model predicted the growth data very well. The poor fit on initial part of the growth is particularly significant for the lowest feeding condition. The reproduction data at the three feeding conditions are well predicted by the model. Similarly, the growth data at the three temperatures correspond well to the model predictions. However, the model's ability to predict the cumulative reproduction data is limited to the lower temperatures, (15 and 20 °C). As shown in Figure 5.10 of the Appendix part, the model strongly over-predicts the cumulative reproduction at 25 °C. Thus, first we estimated T_A by fitting the growth data from the three temperatures and the reproduction data from 15 and 20 °C. Then, using this T_A value we refitted the reproduction data for all the three temperatures by assuming a new yield of egg buffer on assimilates, y_{BA} , value at 25 °C, which is lower than the default value of 0.95. We considered y_{BA} as a new parameter for 25 °C. As shown in Figure 5.3 (bottom), the model now captures the trend in the data and we

estimated $y_{BA} = 0.293$ for the 25 °C condition.

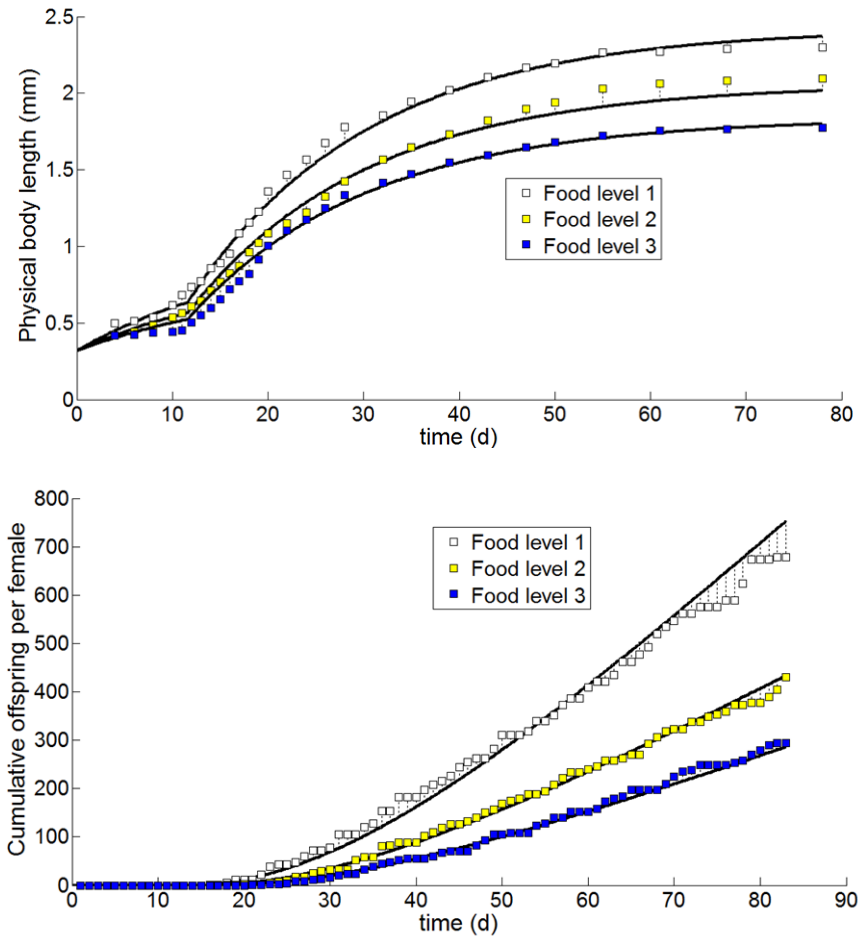


Figure 5.2: The model fitted to the feeding data. Growth pattern at three feeding condition (top) and cumulative reproduction (bottom).

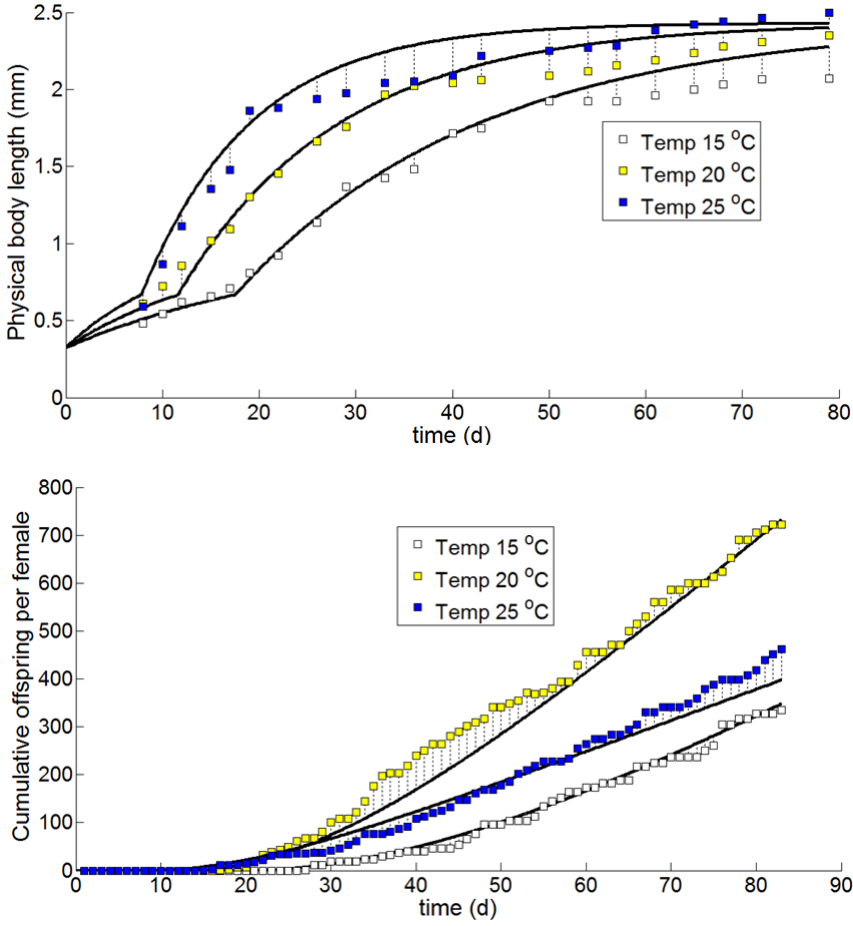


Figure 5.3: Growth pattern at three temperatures (top) and cumulative reproduction (bottom). The deviation of the model from the reproduction data at 25 °C (shown in Figure 5.10 of the Appendix) is corrected by assuming a lower y_{BA} value at this temperature. Time is age post hatching.

Table 5.1: Model parameters and estimated values. Values are obtained/estimated from defaults, directly calculated from measurements, and estimated by optimization. Parameter estimation is performed using maximum likelihood for the feeding and temperature experiments data. The 95% confidences intervals, CIs, of these optimized parameters were derived using profile likelihoods.

Parameter	Unit	Value	Description
Default values [9]			
y_{BA}	-	0.95	yield of egg buffer on assimilates at 15 & 20 °C
y_{VA}	-	0.8	yield of structure on assimilates
Directly calculated from measurements			
W_{B0}	mg	0.00041	initial dry weight of a single egg
δ_M	-	0.30	shape corrector
d_V	mg/mm ³	0.28	dry weight density
Estimated by fitting the model to data			
Using the feeding data			
J_{AM}^a	mg/(mm ² d)	0.0607 (0.0601-0.0650)	Specific assimilation rate at 20 °C
J_M^v	mg/(mm ³ d)	0.066 (0.065-0.072)	specific maintenance costs at 20 °C
κ	-	0.777 (0.774-0.789)	allocation fraction to soma
L_{wp}	mm	0.972 (0.930-1.008)	Length at puberty
f_2	-	0.848 (0.840-0.853)	scaled food level for feeding level 2
f_3	-	0.755 (0.747-0.762)	scaled food level for feeding level 3
s_f	-	0.383 (0.356-0.389)	initial stress factor on food level
T_f	d	12.6 (12.470-12.750)	age post hatching for switching from initial to actual food level at 20 °C
Using the temperature data			
T_A	K	6623 (6580-6684)	Arrhenius temperature constant
y_{BA}	-	0.293 (0.287-0.298)	yield of egg buffer on assimilates at 25 °C

5.3.2 Model simulations

Effect of food on the population growth rate

We calculated the effect of food limitation on the population growth rate of *F. candida* using the Euler-Lotka equation, Equation 5.3. We assumed that the survival is not influenced by food availability (because we did not observe any mortality in our experiment). Figure

5.4 shows this population growth rate at different f values.

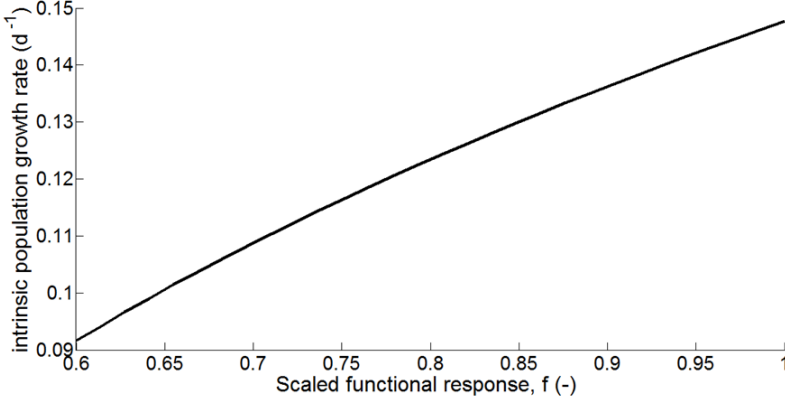


Figure 5.4: The intrinsic population growth rate at different food levels. Food availability is expressed as the scaled functional response, f

Effect of temperature on the population growth rate

Using the DEBkiss model framework and the Arrhenius relationship (Equation 5.1), we can easily estimate body size and reproduction rate at different temperatures. Using the λ and α values we obtained by fitting the survival vs. age curves of Figure 5.11, we can estimate $S(a)$ at 15, 21 and 26 $^{\circ}C$. We can use these temperature dependent vital rates and predict the population growth rate as function of temperature. However, as we mentioned above, the applicability of the Arrhenius correction factor for predicting the temperature dependence of the reproduction rate is apparently limited to a maximum

somewhere between 20 and 25 °C. Thus, in the further analysis of the effect of temperature on the reproduction rate (and estimation of r), we restricted ourselves to a maximum temperature of 21°C, which is found to be the optimum temperature for *F. candida* [17,24].

We predicted the population growth rate, r , for the temperature range from 10 °C to 21 °C. Using the λ and α values, which are obtained by fitting the survival vs. age curves at 15 °C and 21 °C, we derived survival functions for temperature range between 10 °C to 21 °C. Accordingly, for the temperature range between 10-16 °C, we assumed λ and α values similar to 15 °C. Similarly, for the temperature range between 17-22 °C we assumed λ and α similar to the values obtained from 21 °C survival curve. Putting all these temperature dependent vital rates into the Euler-Lotka equation (Equation 5.3), we predicted the population growth rate as function of temperature (Figure 5.5).

The combined effect of food and temperature on population growth rate

The combined effect of food availability and temperature is shown as a surface plot for the intrinsic population growth rate in Figure 5.6, while Figure 5.7 shows the contour plot of the intrinsic population growth rate. Figure 5.8 illustrates the differences of the two population growth rates for different temperature and food density interactions (Δr value in Equation 5.5).

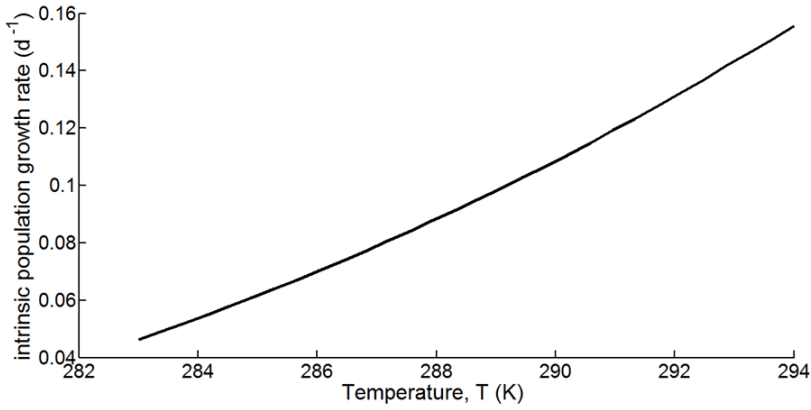


Figure 5.5: The intrinsic population growth rate as function of temperature. Considering the limitation of the model to capture the effect of temperature at the highest temperature, our simulation is only for the temperature range below the optimum (i.e. 21 °C)

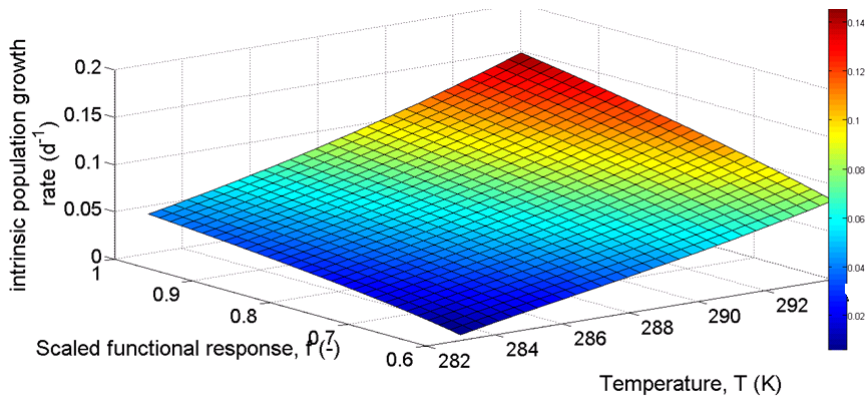


Figure 5.6: The combined effect of food availability and temperature on the intrinsic population growth rate of *F. candida* presented as surface plot

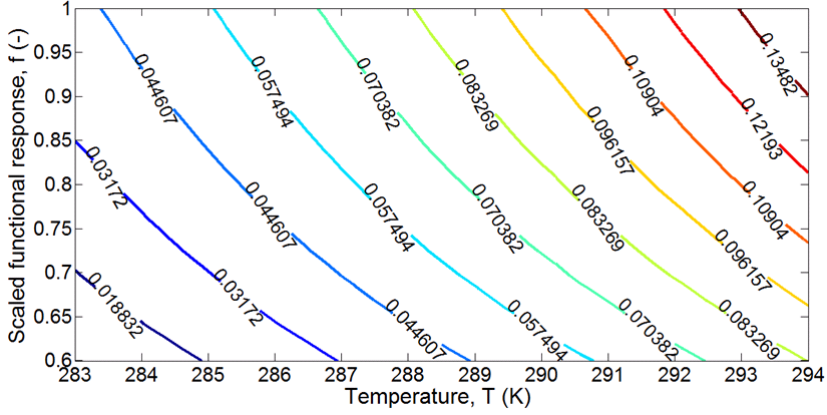


Figure 5.7: Iso-lines of the intrinsic population growth rate (day^{-1}) as function of temperature (in Kelvin, in X -axis) and food availability (expressed as scaled functional response, f in Y -axis).

5.4 Discussion

5.4.1 Model assumptions and their validity

The DEBkiss model

The DEBkiss model is a simplified version of the standard DEB animal model. The main simplification of the model is that it does not have a reserve compartment and maturity is not considered as a state variable [9]. The validity of these simplifications for our case is indicated by the excellent fit on the growth curves (a substantial reserve compartment would lead to a different shape at different food levels) and the rather constant size at first reproduction (which indicates that maturity does not need to be followed explicitly).

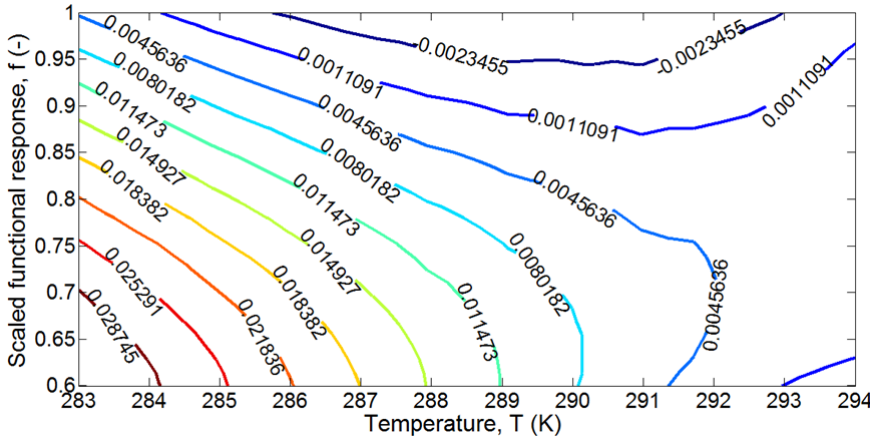


Figure 5.8: A contour plot showing the difference between the change in population growth rate calculated by adding up the change in population growth rates due to food limitation and sub-optimal temperature independently and the change in population growth rate due to the combined effects of sub-optimal temperature and food limitation. Iso-lines with a negative value indicates the combined effect is less than additive and positive indicates more than additive.

Deviation from the von Bertalanffy growth pattern

At constant food conditions, DEB models predict that the growth of animals follows the von Bertalanffy curve as long as the animal does not change in shape over growth [3]. However, at early age, the growth of *F. candida* deviates from this expectation. The age-dependent switch in feeding that we assumed here provides a good fit to the body size data for all treatments. A possible explanation for this initial period of low feeding might be the need for development

of a fully functional gut flora to aid digestion, or the need for learning/adaption of a new feeding method and environment. Another potential explanation for this initial slow feeding might be behavioral. The age based dispersion studies by Johonson and Wellington [18] on *F. candida* shows that feeding behavior differs between early stage juveniles and other groups. In their study, they found that smaller-sized juveniles (< 0.6 mm) spend less time around food sources than the medium-sized ones. During our experiment, we also observed that at their early stage, the juveniles spend most of their time spreading around and spend much less time around food. The significant misfit for the lowest feeding conditions might be because of the intermittent feeding regime used for experiment that the animals are adapting to.

Correcting parameters for the effect of temperature

As explained in Section 5.4, we included the effect of temperature by scaling all rate parameters using a correction factor. We therefore applied the Arrhenius correction factor (F_T , Equation 5.1) to the specific assimilation and maintenance rates, as well as the duration of the initial slow-feeding stage (T_f). This assumption captured the temperature-dependent growth of *F. candida* well at all three experimental temperatures. As shown in Figure 5.3, at 25 °C, *F. candida* grows faster and it has the highest body growth rate. However, the assumption failed to capture the reproduction data at 25 °C. At this

temperature, the model predicted the highest reproduction rate, but the actual data showed a much lower reproduction rate than at 20 °C (see Figure 5.10 in the Appendix part). Thus, the applicability of the Arrhenius correction factor (Equation 5.1) for predicting the temperature dependence of the reproduction rate is limited for a range of temperatures lower than 25 °C. Previous studies showed the same trend for the effect of temperature on growth and reproduction of springtails. For example, Choi et al. [19], studied the influence of temperature on the biology of *Paronychiurus kimi* (Lee), and found that, though *P. kimi* grows faster for a temperature range of 25-30 °C, the reproduction of the organism is inhibited. The authors explained the inhibition of reproduction at this high temperature from an energy allocation point of view. They hypothesize that the higher respiration rate of the springtail at high temperatures requires more energy and thereby reduces the energy available for reproduction. However, a higher respiration rate also reduces the energy available for growth, and this hypothesis does not explain why only reproduction is affected and not growth. Both *F. candida* and *P. kimi* grow faster in the temperature ranges at which their reproduction is inhibited. From a DEB context, we might speculate that, at higher temperatures, the organisms have to spend more energy on maturity maintenance, or that the conversion of the energy available for reproduction to eggs

is less efficient. As presented above, we assumed the latter case (i.e., a y_{BA} value lower than 0.95 at 25 $^{\circ}\text{C}$) and refitted the reproduction data of the three temperatures simultaneously. As shown in Figure 5.3, this new y_{BA} value fixed the discrepancy observed in Figure 5.10 and the model now captures the trend in the data. Here it is worth mentioning that our hypothesis for this deviation is speculative, and requires further dedicated study.

5.4.2 Effects of food density on growth, reproduction and population dynamics of *F. candida*

The effect of food density on the individual life-history traits was modeled by decreasing the scaled functional response, f . This lowers the assimilation flux, and thereby the amount of energy available for growth and reproduction. As shown in Figure 5.2, food limitation is shown to have highly predictable effects on growth and reproduction. From the data and the model fits it is clear that food limitation affects both the growth rate and ultimate size of *F. candida*. There are few studies on the effect of food availability on the vital rates of *F. candida*. For example, Hafer et al [20] studied the effects of food density on age at maturity and reproductive output across three generations in *F. candida*. In general, their findings showed that, at lower food density, the age at maturity increases and the egg production rate

decreases. Our findings are consistent with their observation; the increased age at maturation is fully explained by a slower growth rate at low food, coupled to a constant size for maturation.

These effects on individual-fitness influence the population growth rate of *F. candida*. As depicted in Figure 5.4, the intrinsic population growth rate shows an almost linear increase with the scaled functional response, f . This population-level influence of food density is due to its direct and indirect effects on the reproduction rate $R(a)$. Less food immediately means less energy available to produce offspring. However, less food also means less growth, and hence a delay in the start of reproduction.

One interesting findings from the study is the quantitative relationship between the actual amount of food available for the organisms and the scaled functional response. Despite the fact that at the lower feedings conditions the food was available only for $\frac{1}{3}$ and $\frac{1}{5}$ of the total time, we estimated $f \approx 0.85$ and $f \approx 0.76$ on the life-history data (Table 5.1), whereas values around 0.33 and 0.2 might have been expected. This implies that the animals that feed only every 3rd or 5th day ate much more during those days than the animals in the ad libitum treatment. This is consistent with the argument made by Kooijman [3]: when starved animals are fed, they often ingest at a higher rate for a short time.

5.4.3 Effects of temperature on growth, reproduction and population dynamics of *F. candida*

Our simulation results show that the body growth rate of *F. candida* increases with temperature. The stimulating effect of temperature on the body growth rate is caused by an increase of the specific assimilation and maintenance rates, and a decrease in time needed to adapt to the food situation. As shown in Figure 5.3, the effect of temperature is limited only to the growth rate of the body; in contrast to the effect of food limitation, temperature hardly affects the ultimate size, which is consistent with the model predictions. The effect of temperature on the reproduction rate is due to its direct influence on the assimilation rate and the indirect effect on the growth rate.

Although the effects of temperature on individual vital rates of *F. candida* were reported in different works (e.g., [15, 17, 21, 22, 25]), these cited works do not provide a mechanistic explanation for these effects. The present study proved that DEBkiss is a suitable analytical tool to unveil the processes responsible for the effects of temperature on the vital rates and provide an understanding of the underlying mechanisms. As discussed above and shown in Figure 5.3, the model well predicted the growth and reproduction data (at least at 15 and 20 °C). We also tested the credibility of the model by comparing the model prediction against our observation for derived parameters,

i.e., age at first reproduction and embryonic period, for the three test temperatures. The model predicted that *F. candida* reach maturity and start laying eggs at the age of 19, 15 and 11 days at 15, 20 and 25 °C, respectively; which are earlier than our observation and results reported by Stam et al. [22], Gregoire–Wibo and Snider [17], and Johnson and Wellington [21]. The continuous reproduction assumption we used in the model is the possible explanation for these discrepancies. In the model we assumed that, immediately when the animals reached maturity, they start allocating energy to reproduction and produce egg continuously. However, in reality they reproduce in discrete events (spawning) and produce a clutch of eggs. Individual differences and age inconsistency of the animals used for the study can also explain some of the discrepancy. The constant body length at puberty, L_{wp} , we assumed for all three temperatures might also contribute to the relatively higher differences observed at 25 °C. In the model we used L_{wp} , which is estimated from the feeding experiment data. As explained above, this temperature might have additional stress on the reproduction process of the organism, for example increased maturity maintenance or reduced egg conversion efficiency. Thus despite of reaching L_{wp} early, the organism might not start allocating energy to reproduction, thereby delaying the start of reproduction.

According to our observations, the average time it takes the eggs to hatch at 15, 20 and 25 °C is 19, 11 and 7 days, respectively. These values are similar to the result reported by Snider and Butcher [24]. However, the results from our model are roughly a factor of two lower than these average values; i.e., 8.8, 5.4 and 3.4 days at 15, 20, and 25 °C respectively. Our prediction is even lower than the minimum embryonic development period of 5 days reported by Green [23] at 25 °C. This discrepancy might be explained by a slower utilisation of egg buffer by the developing embryo than predicted from juvenile/adult performance (a similar discrepancy was observed in the pond snail, [9]).

As depicted in Figure 5.5, the intrinsic population growth rate of *F. candida* increases with temperature. Temperature influence the population growth due to the direct and indirect effects of temperature on the reproduction rate $R(a)$. The effect of temperature on the population growth rate of *F. candida* is also reported by a number of authors. For instance, the values of r reported by [17] and Subagja [26] are comparable to our model predictions for similar temperatures.

5.4.4 Combined effects of food density and temperature on population dynamics of *F. candida*

In the natural environment, stressors do not occur alone, but rather in combination, and they can interact and influence the dynamics of populations. For instance, many populations experience strong seasonal fluctuations in temperature and food availability. One of the advantages of theory-based models is that they allow the integration of information from empirical and field studies involving different interactions of organism and environmental factors, and thereby help contribute to predictions for new situations [7]. Using our model, we are able to predict the combined effect of temperature and food availability on the population dynamics of *F. candida* based on two separate experimental studies (i.e., effect of temperature and food availability). The model simulation result show that for temperatures above 10 °C, the population growth rate of *F. candida* is always positive, even under rather extreme food limitation (Figure 5.7). This suggests that *F. candida* is quite able to cope with stressors arising from temperature change and food limitation. Even though we could not obtain data to verify our predictions on the combined effects of the two stressors, the ability of the organism to resist different natural stressors is confirmed by empirical studies. For example, Bayley and Holmstrup [27] reported that the animals are tolerant to drought

stress and capable of living under extremely dry conditions. Hilligsøe and Holmstrup [28] further studied the effects of starvation on drought tolerance and show that *F. candida* is resistant to starvation and its drought tolerance ability is not affected food limitation.

The inconsistent trend of the interaction between food density and temperature is quite interesting. As illustrated in Figure 5.8, the effect arising between the two factors produced all the three possible cases (i.e., additive, more than additive and less than additives). However, for most of the cases the interaction is more than additive as the combined effect is greater than the sum of their individual effects. As shown in Figure 5.8, for the temperatures below 15 °C and food limited, the combined effect of the two stressors always results in an effect greater than the sum of the individual effects.

Considering the seasonal and/or spatial variability of temperature and food availability in the natural environment, this study is useful to understand the ecology of *F. candida*. The work also sheds light on the applicability of the DEBkiss model to interpret the interaction and combined effects of different environmental stressors on the life-cycle and population dynamics of springtails. For example, the model can easily be extended to assess and predict the combined effects of chemicals and environmental stressors, which is presented in the next chapter of the thesis. Thus, it can be a potential tool for the

mechanistic understanding of the combined effects of the stressors, and provide educated extrapolations to different conditions, which is a major challenge in ecotoxicology and ecological risk assessment (ERA) of chemicals. *F. candida* is a standard test organism for terrestrial risk assessment of chemicals, and a parameterized model for this species will thus be an important contribution for the application of the model in ERA of chemicals.

5.5 Conclusion

In this paper, we parameterized and implemented a simplified DEB model (DEBkiss) to explore the effects of food availability and temperature on the vital rates and population growth of *F. candida*. The DEBkiss model framework is transparent and applies an explicitly-visible mass balance to mechanistically describe the metabolic processes of an organism. We showed here how the various model parameters can be obtained by performing simple life-cycle experiments and observing standard end-points at different environmental conditions. However, here it is worth mentioning that the design of the experiments should be an integral part of the modeling process. Our results show that the life history of *F. candida* is influenced by both food limitation and temperature in a predictable manner. We also showed that food limitation and temperature can have different of

interactions on the dynamics of *F. candida*. Verifying the model simulation results based on a study of the combined effect of food limitation and temperature would be interesting to further validate the practical applicability of the model.

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Appendix

Conceptual framework and assumption of the model

The full DEBkiss model we departed from is schematically shown in Figure 5.9. The model follows mass fluxes as dry weight and uses

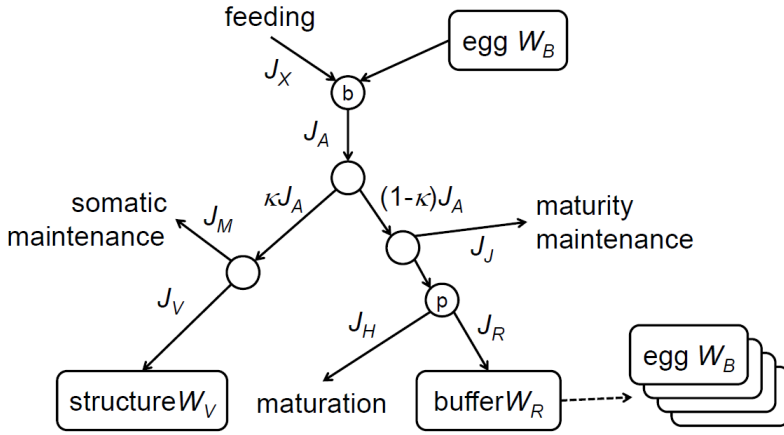


Figure 5.9: Schematic diagram of the mass flows in the full DEBkiss model extended with the maturation flux (J_H), feeding, mass of assimilates buffer in egg (W_B) and mass of reproduction buffer in adult (W_R), (Re-used from [9] with permission).

three state variables to describe the dynamics of the mass fluxes. The three state variables (shown as rectangles in Figure 5.9) that are involved in the model are: egg buffer W_B , structural body mass W_V and reproduction buffer W_R . In this study, we followed only W_V as state variable. The full DEBkiss model involves seven mass fluxes, here we considered only the assimilation flux, J_A ; the flux

for structural growth, J_V ; the flux into the reproduction buffer, J_R ; somatic maintenance flux, J_M and maturity maintenance flux, J_J .

Tables 5.2 and 5.3 summarize the assumptions that the model is based on.

Model equations and derivations Differential equations

We followed the structural body mass, W_V , as state variable and mathematically represented by a differential equation as follows:

$$\frac{d}{dt}W_V = J_V \quad \text{with } W_V(0) \approx 0 \quad (5.6)$$

Mass fluxes

Five mass fluxes are involved in the model: assimilation flux, J_A ; the flux for structural growth, J_V ; the flux into the reproduction buffer, J_R ; somatic maintenance flux, J_M and maturity maintenance flux, J_J . These fluxes are mathematically described as follows.

Assimilation flux (J_A) The assimilation flux is assumed to be proportional to the surface area of the animal, and thus to L^2 . The maximum specific assimilation rate (J_{Am}^a) is considered as proportionality constant:

$$J_A = f J_{Am}^a L^2 \quad (5.7)$$

Where f is the scaled functional response. Note that $L = \left(\frac{W_V}{d_V}\right)^{1/3}$.

Maintenance (J_M) and growth (J_V) The assimilated flux, J_A

Table 5.2: The list of assumptions that leads to the DEBkiss model.

- There are three types of biomass: food, assimilates and structural body components. Each type has a constant composition. They can be converted in each other with a certain constant efficiency. The state variables of the organism are the masses of the structural body, the reproduction buffer for adults, and the egg buffer for embryos. Total body mass is the sum of structure and reproduction buffer. The reproduction and egg buffer consist of assimilates.
 - The animal has three life stages: an embryo that does not feed but utilises a buffer in the egg, a juvenile that feeds but does not reproduce, and an adult that reproduces. The embryo starts with a buffer of assimilates in the egg and negligible structural mass. The first transition (‘birth’) is triggered by the depletion of the egg buffer, and the second transition (‘puberty’) when a critical structural body weight is reached.
 - The maximum feeding rate is proportional to the surface area of the animal. The animal is either searching for food or handling it (with constant handling time), leading to a hyperbolic functional response in the food density (Holling type II).
 - Food is instantly translated into assimilates that are directly used to fuel metabolic processes. Embryos assimilate their egg buffer at the maximum rate for their size.
 - The flow of assimilates is split into a constant fraction κ for maintenance and structural growth (the soma), and $1 - \kappa$ for maturation, maturity maintenance and reproduction. From the κ flow, maintenance costs are paid first. Only structural biomass requires maintenance, which is proportional to its volume. The remainder of this flow is used for growth (with certain efficiency).
-

splits into a constant fraction κ for maintenance and structural growth (the soma), and $1 - \kappa$ for maturation, maturity maintenance and re-

Table 5.3: The list of assumptions that leads to the DEBkiss model.

- For adults, the $1 - \kappa$ flow is used to fill the reproduction buffer and to pay for maturity maintenance. Maturity maintenance is paid first. The remainder goes to the reproduction buffer. For embryos and juveniles, all of the assimilates in this flux are burnt to increase and maintain complexity of the organism. At spawning events, the contents of the reproduction buffer are converted into eggs. The part of the buffer that was insufficient to create a single egg remains in the buffer. Transformation of buffer to egg comes with a certain (generally high) efficiency. Maturity maintenance is proportional to structural volume up till puberty. After puberty, maturity maintenance is fixed to the level at puberty.
 - If feeding is insufficient to pay somatic maintenance costs, the organism first diverts energy from the $1 - \kappa$ flux of assimilates and from the reproduction buffer. If that is insufficient, structure is converted into assimilates to pay maintenance. Under starvation, maturity maintenance is paid from the reproduction buffer as long as there is something in the buffer. Maturity maintenance is not paid from structure.
-

production. The maintenance flux is proportional to the structural volume, L^3 . The volume-specific maintenance cost (J_M^v), a primary parameter, is used as proportionality constant.

$$J_M = J_M^v L^3 \quad (5.8)$$

Maintenance costs are paid first and thus, the flux for structural

growth (J_V) can be specified as:

$$J_V = y_{VA}(\kappa J_A - J_M) \quad (5.9)$$

where y_{VA} is the yield of structural biomass on assimilates.

Reproduction flux (J_R) The $1-\kappa$ fraction of the assimilated flux is used for reproduction in adults and for maturity maintenance. This flux is used for the maturation process until the animal reaches puberty. After the animal reaches maturity it is directed to reproduction, J_R and maturity maintenance, J_J . Maturity maintenance is also paid by juveniles.

$$J_R = \begin{cases} (1 - \kappa)J_A - J_J & \text{if } W_V \geq W_{Vp} \\ 0 & \text{if } W_V < W_{Vp} \end{cases} \quad (5.10)$$

Maturity maintenance flux (J_J) The maturity maintenance flux, J_J , is proportional to the structural volume, L^3 , but fixed at puberty. The volume-specific maturity cost (J_J^v) is used as proportionality constant.

$$J_J = \begin{cases} J_J^v L_p^3 & \text{if } W_V \geq W_{Vp} \\ J_J^v L^3 & \text{if } W_V < W_{Vp} \end{cases} \quad (5.11)$$

J_J^v is a primary parameter but here we set it to a 'suggested value',

by assuming a link to somatic maintenance (see [9]).

$$J_J^v = \frac{1 - \kappa}{\kappa} J_M^v \quad (5.12)$$

Links between state variables and measured quantities

The model output for body size is dry weight. In this study, however, we measured body length in the various treatments. The structural weight, W_V , can be translated to measured physical body length, L_w , by assuming that the organism does not change in shape during growth. Defining two new parameters, the shape correction (δ_M) and the density of biomass (d_V), measured physical body length, L_w , and the structural body length, L can be calculated as

$$L_w = \frac{1}{\delta_M} \left(\frac{W_V}{d_V} \right)^{1/3} \quad (5.13a)$$

$$L = \left(\frac{W_V}{d_V} \right)^{1/3} \quad (5.13b)$$

Size and age at birth

Structural body size at birth, (W_{Vb}) and age at birth, (t_b) can be calculated using Equation 5.14 (see [9] for the details of the assumptions

and derivations):

$$W_{Vb} = W_{B0} y_{VA} \kappa \quad (5.14a)$$

$$t_b = \frac{3W_{B0}^{1/3} d_V^{2/3}}{J_{Am}^a (y_{VA} \kappa)^{2/3}} \quad (5.14b)$$

The diameter of the egg is used to estimate the volume of the egg (by assuming the egg is a perfect sphere), and this volume converted to dry weight to yield the egg weight W_{B0} . If we assume a negligible somatic maintenance cost for a newly hatched juvenile, the body mass at birth W_{Vb} can easily be calculated from egg weight (Equation 5.14a). This body mass at birth is used as initial value for fitting the growth and reproduction data.

The list and description of the parameters in the model are presented in Table 5.4.

Initial fit of the model to reproduction data

We included the effect of temperature in the DEBkiss model by scaling all rate parameters using the Arrhenius correction factor, F_T , which is calculated using Equation 5.1. However, our initial fit indicated that the model strongly over-predicts the cumulative reproduction at 25 °C. As shown in Figure 5.10, the model captures the trend of the cumulative reproduction data at 15 and 20 °C; however at 25 °C, there is a clear misfit between the model prediction and data. As ex-

Symbol	Explanation	Dimension	Sugg. value
Primary parameters			
J_{Am}^a	Maximum area-specific assimilation rate	$m_a/(l^2t)$	—
J_M^v	Volume-specific maintenance costs	$m_a/(l^3t)$	—
W_{B0}^M	Assimilates in a single freshly-laid egg	m_a	—
W_{Vp}	Structural body mass at puberty	m	—
y_{BA}	Yield of egg buffer on assimilates	m_a/m_a	0.95 mg/mg (dwt)
y_{VA}	Yield of structure on assimilates	m/m_a	0.8 mg/mg (dwt)
κ	Fraction of assimilation flux for soma	—	0.8
Conversions			
d_V	Dry-weight density of structure	m/l^3	0.1 mg/mm ³
δ_M	Shape correction coefficient	—	—
Fluxes, states and forcings			
J_A	Mass flux for assimilation	m_a/t	—
J_M	Mass flux for somatic maintenance	m_a/t	—
J_R	Mass flux to reproduction bufeer	m_a/t	—
J_V	Mass flux for structure	m/t	—
J_J	Mass flux for maturity maintenance	m_a/t	—
J_H	Mass flux for maturity	m_a/t	—
W_V	Mass of structural body	m	—
Other output and intermediate parameters			
f	Scaled functional response (0-1)	—	—
L	Volumetric body length	l	—
L_w	Physical body length	l	—
r_B	Von Bertalanffy growth rate constant	$1/t$	—
R	Continuous reproduction rate	$\#/t$	—
t_b	Time between egg laying and birth	t	—
W_{Vb}	Structural body mass at birth	m	—
W_w	Physical body weight (total)	m	—

Table 5.4: Explanation of symbols, with dimensions given in mass (m for body and m_a for assimilates), length (l_e for environment, l for organism), numbers ($\#$), time (t). Suggested values for the yields (apart from y_{AV}) based on the typical values in [9].

plained in Section 5.3.1 and also shown in Figure 5.3 (bottom), this misfit was improved by using a different y_{BA} at 25 °C, which is lower than the suggested default value of 0.95. **Survival Model**

We assumed a Weibull survival function of $S(a) = \exp(-\lambda a^\alpha)$. We estimated λ and α at three temperatures by fitting the survival data obtained from Snider and Butcher [24]. Table 5.5 shows the estimated values for α and λ at 15 °C, 21 °C and 26 °C; while Figure 5.11 shows the model fit to the survival data for the three temperatures.

We used the MATLAB Curve Fitting Toolbox, which assumes least

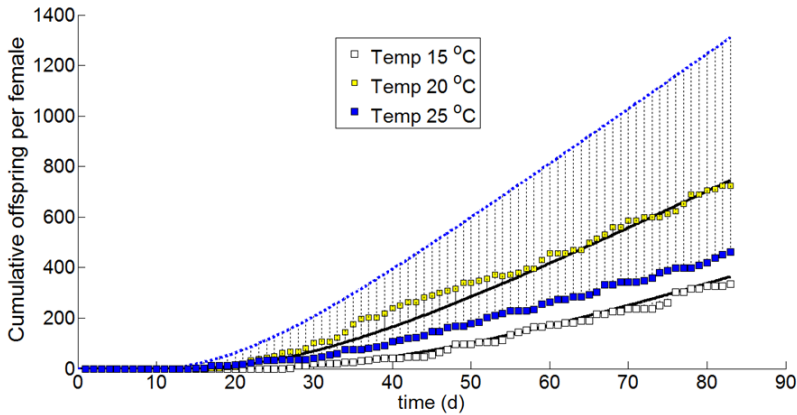


Figure 5.10: Fits of the DEBkiss model to the reproduction data for three temperatures. At 25 °C, the model prediction is much higher than observation (blue broken lines and blue squares). For the fit we assumed $y_{BA} = 0.95$ for all three temperatures. The fit is improved using a lower y_{BA} value at 25 °C (Figure 5.3).

squares error minimization to estimate the parameters and their confidence of intervals.

Temperature	$\lambda(CI)$	$\alpha(CI)$
T = 15 °C	$4.98 \cdot 10^{-8}$ ($7.57 \cdot 10^{-9} - 9.2 \cdot 10^{-8}$)	2.97 (2.85-3.1)
T = 21 °C	$3.14 \cdot 10^{-8}$ ($-1 \cdot 10^{-8} - 7.3 \cdot 10^{-8}$)	3.414 (3.23-3.61)
T = 26 °C	$1.36 \cdot 10^{-6}$ ($-2.25 \cdot 10^{-7} - 3 \cdot 10^{-6}$)	3.12 (2.85-3.38)

Table 5.5: Parameter estimates for the fits to survival data at three temperatures: see Figure 5.11

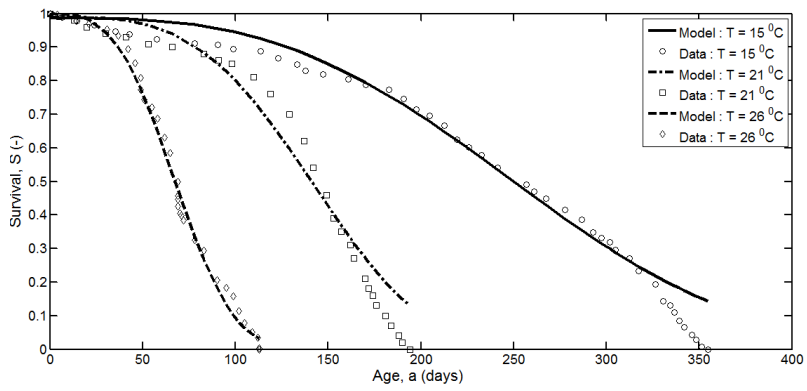


Figure 5.11: Fits of the survival model to data obtained from [24] for three temperatures.

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Chapter 6

Simplified energy-budget model for the mechanistic interpretation of the combined effects of temperature and cadmium on *Folsomia candida*

Prepared as manuscript: Hamda N. T., Jager T., Laskowski R. Simplified energy-budget model for the mechanistic interpretation of the combined effects of temperature and cadmium on *Folsomia candida*

Abstract

With the current focus on predicting population-level effects of chemicals, a tendency has developed to integrate population models in the risk assessment process. The purpose of these models is to extrapolate organism-level effects observed in the laboratory to population-level impacts. Currently, individual-level effects are assessed using dose-response relationships derived from standard ecotoxicological tests. However, extrapolating these effects to population is flawed because typical approaches for dose response analysis do not take into account the factor of time and other environmental stressors that can influence effects of chemical at the individual-level and its consequence to the population. In this study, we applied the simplified DEB model (DEBkiss) to explore the combined effects of Cd and temperature on the vital rates of the springtail *Folsomia candida*. We integrated the DEBkiss model with the Euler-Lotka equation to extrapolate the combined effects of the two stressors to the population-level. At the individual-level, the DEBkiss model was able to capture the effects of Cd on *F. candida* at 15 and 20 °C, but due to the higher mortality and low reproduction rate, we could not obtain a reliable fit at 25 °C. Using the model outputs, we estimated EC₂₅ and EC₅₀ as function of time for growth and reproduction, respectively. These estimates were lower at 15 °C, which indicates that the magnitude of effects

of Cd on vital rates of *F. candida* is higher at 15 °C than at 20 °C. The response of *F. candida* populations to increasing concentrations of Cd was predicted using the Euler-Lotka equation. We found that the population growth rate of *F. candida* decreases at a higher rate at 15 °C than at 20 °C with increased cadmium concentrations. We conclude that extrapolation of effects from the current standard of 20 °C in toxicity tests to individuals and population living in different temperature conditions might lead to erroneous conclusions. Our study demonstrated that energetic-based models, like DEBkiss, hold a great potential for the mechanistic interpretation of the combined effects of chemicals and environmental stressors on individuals. We also showed the advantages of these mechanistic models for developing population models to extrapolate effects to the population-level.

Key words: DEB, DEBkiss, toxicokinetics, toxicodynamics *Folsomia candida*, environmental stressors, cadmium

6.1 Introduction

The protection goals for ecological risk assessment are at higher biological organization; e.g. populations or entire ecosystems. However, it is difficult or even impossible to study the effects of toxicants at the population or ecosystem level due the complexity of such experiments, and other technical, ethical and legal issues. Thus, effects of chemicals are usually assessed from laboratory experiments with individuals of selected species. The effects are determined by following individual end-points like body size, reproduction rate and short-term survival at different levels of chemical exposures or doses. However, from an ecological perspective, these individual-level end-points from a single species provide limited information to assess toxicity at the population or ecosystem level. Thus, linking these observations to effects at higher levels of biological organization is the central challenge of ecotoxicology [1] and increasingly, ecotoxicologists are turning their attention to the relationships between effects of chemicals at higher biological organization and the effects on individual life-history traits that contribute to it [2].

This shift from extrapolating effects of toxicants from individuals to higher levels of biological organization is receiving increasing emphasis by regulatory agencies such as the US Environmental Protection Agency (US EPA) and the European Union [3, 4]. To address these

goals, population models have been advocated to integrate population dynamics in the risk assessment process [5–10]. The aim of these models is to extrapolate organism-level effects observed in the laboratory to population-level impacts. The application of population models has great potential for adding value to ecological risk assessment [5, 11], and the case studies presented by Akçakaya et al. [10] demonstrated the diverse array of applications of population models as a tool for ecological risk assessment of chemicals.

Usually, population models are parameterized using individual-level endpoints from laboratory toxicity tests, analyzed by fitting empirical models to concentration-effect relationships to the observations at the end of the test. This procedure, however, hampers the potential relevance of population modeling as a tool in the ERA process due to the lack of a mechanistic basis [12, 13]. Such a mechanistic basis is particularly important for the extrapolation of effects.

In ecotoxicology, toxicity tests are usually highly standardized and conducted under constant (presumable favorable) environmental conditions, in an attempt to isolate the effects of the chemical stressor(s) from all other environmental influences. However, in their natural environment, organisms rarely experience optimal conditions, and these conditions are rarely constant for long. Quite the opposite, organisms are regularly forced to cope with either suboptimal conditions or se-

vere environmental stress for most of their life spans [14, 15]. Time is another important factor that is not usually considered in standard bioassays. To exert their effects, chemicals must be transported from the environment to target sites, and should accumulate there to concentration levels that drive the toxic effects. The chemical specific nature of these processes is completely ignored, as standard tests are conducted for the same specified duration for all chemicals. A final limitation of standard tests is that they focus on the effects on a single endpoint only, often the endpoint that is considered to be demographically sensitive. However, effects on the most sensitive life stages do not necessarily drive population-level effects [?]. Hamda et al. [16] showed that a population-level interpretation of individual-level experimental results required information on two fronts; first, on how the toxicant affected the life stage under study, and second, on how sensitive the population-end point (e.g., population growth rate, r) was to changes in that particular life stage. Forbes and Calow [17] also found inconsistent relationships between the effects of a toxicant on the population growth rate (r) and on individual life-history traits contributing to it.

The limitations mentioned above can be overcome, and a reliable population model can be developed, by formulating a mechanistic effect models at the individual level to interpret and predict organism-level

effects of chemicals. Such mechanistic effect models should preferably be derived from a general energetic theory, and the Dynamic Energy Budget (DEB) theory is currently the most comprehensive and best-tested theory in this field. In this study, we applied a simplified DEB model (DEBkiss, [18]) for the mechanistic interpretation of the combined effects of temperature and cadmium in food on vital rates of the springtail *F. candida*, and we integrated the model using the Euler-Lotka equation to predict the effects on the population growth rate of the organism. The model we used here is an extension of the basic DEBkiss model, which was calibrated for our model organism (Chapter 5). In this study, we include a toxicokinetic (TK) and toxcodynamic (TD) model in this framework. Experimental studies were conducted in three temperature regimes and five cadmium (Cd) concentrations to parameterize the TK and TD. The parameterized model was subsequently applied to explore the effects of temperature and time on the classic summary statistic, EC_x for growth and reproduction (the concentration of Cd at which growth and reproduction were reduced to $x\%$ of that of the control). Using the Euler-Lotka equation, we derived a dose-response (or a concentration-effect) relationship between Cd and the intrinsic population growth rate of *F. candida* at different temperatures.

6.2 Materials and Methods

6.2.1 Data collection

Test animals

A laboratory culture of *F. candida*, which had been kept for more than 10 years at the Institute of Environmental Sciences of the Jagiellonian University, Poland, was used for this experiment. The animals were maintained as stock cultures in plastic boxes filled with moist plaster of paris, mixed with charcoal, at a constant temperature of 20 °C and with dried baker's yeast (Dr. Oetker) as food. Prior to the experiments adult animals were transferred to plastic boxes of 10 cm in diameter with plastic screw top lids and filled with moist plaster of paris to lay eggs. After 7 days, the adults were removed and the eggs were allowed to hatch. Emerging juveniles of age between 1 and 3 days were used in the experiments.

Preparation and contamination of the food

The test animals were exposed to Cd by feeding a yeast paste (commercial bakers' yeast Dr. Oetker) contaminated with $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$. We firstly dried the yeast at 105 °C for 24 hours. We prepared a Cd solution of desired concentration (prepared on the basis of dry weight of the yeast) and mixed with dry yeast using the Electromagnetic

stirrer WIGO.

Experimental design

Toxicity tests were carried out using four treatments and a control at three constant temperatures. The food was freshly prepared each week and old food was removed. Nominal Cd concentrations of 100, 500, 1500 and 2000 (in mg /kg dry yeast) were used as treatment. We were mainly interested in sub-lethal effects, so the choice of these concentrations was based on concentration-effect relationships reported by Crommentuijn et al. [19,20] and Fountan and Hopkin [21]. The experiments were carried out in plastic containers (3.5 cm in diameter) with plastic screw top lids. Freshly hatched 1-3 days old juveniles were individually kept in plastic vessels (diameter 3.5 cm) filled with moist plaster of paris mixed with charcoal. For each treatment, 10 replicates were prepared. The experiments were conducted in three climate chambers kept constant at 15, 20, and 25 °C. Relative humidity of the test containers was maintained at 100% during the experiment by spraying the plaster of paris with distilled water regularly. The contaminated yeast paste (volume of 120 μ l) was first placed on transparent circular plastic plates of diameter 1 cm using a HTL Pipette. The plastic plates were placed at the center of each test container ensuring that the mixture did not spread on to the plaster of Paris.

Body length and reproduction of the individuals were observed over 12 weeks. Digital image processing equipment was used to record individual body lengths (recorded every day for the first 3 weeks, every 2-4 days until the seventh week, and once per week thereafter until the end of the experiment). Body lengths (i.e., the distance from the posterior end of the abdomen to the anterior end of the head between the antennae) were measured using the free image analysis software ImageJ (v.1.47p); (National Institute of Health, USA, <http://imagej.nih.gov>). Individuals were monitored every day for egg production using a microscope. When eggs were observed, the individuals were transferred (since this species eats their eggs) to new test containers with the same conditions, and observations continued. The eggs in the old test containers were photographed using a high-resolution camera (EOS 1000D with 60 mm Macro lens) and the eggs were counted using ImageJ (v.1.47p) and manually. As the species usually lays eggs inside cracks of plaster of paris and in cracks between the test plastics and the plaster of paris, we also followed the hatched juveniles for verification. Beside these continuous observations, the diameter of the eggs at first reproduction was measured, and the age at which the first eggs were produced was recorded.

6.2.2 Brief description of the DEBkiss Model

The model we used in this study was developed by extending the simplified DEB model, DEBkiss [18]. This specific model has been described in detail by Jager et al. [18], and here we will briefly discuss the most important principles of the model (detailed model equations can be found in the supplementary material). The DEBkiss model describes how food is used to fuel the metabolic processes of growth, development, maintenance and reproduction. It follows mass fluxes as dry weight, as depicted in Figure 6.1

6.2.3 Extension for juvenile stage food limitation

As presented in Section 5.2.4 of this thesis, the growth of *F. candida* deviates from the von Bertalanffy growth curve, as predicted from the DEBkiss model, at its early stage. As discussed in the previous chapter of the thesis, we accounted juvenile stage food limitation by introducing a stress factor on feeding for the early-juvenile period. This model extension is also applied here (see Section 5.2.4 for detailed description of the model extension).

6.2.4 Modelling effects of toxicants

In the DEB context, the effects of toxicants on vital rates of an organism occur by altering one or more of the metabolic processes described in Figure 6.1 [22]. The severity of the effect on the metabolic pro-

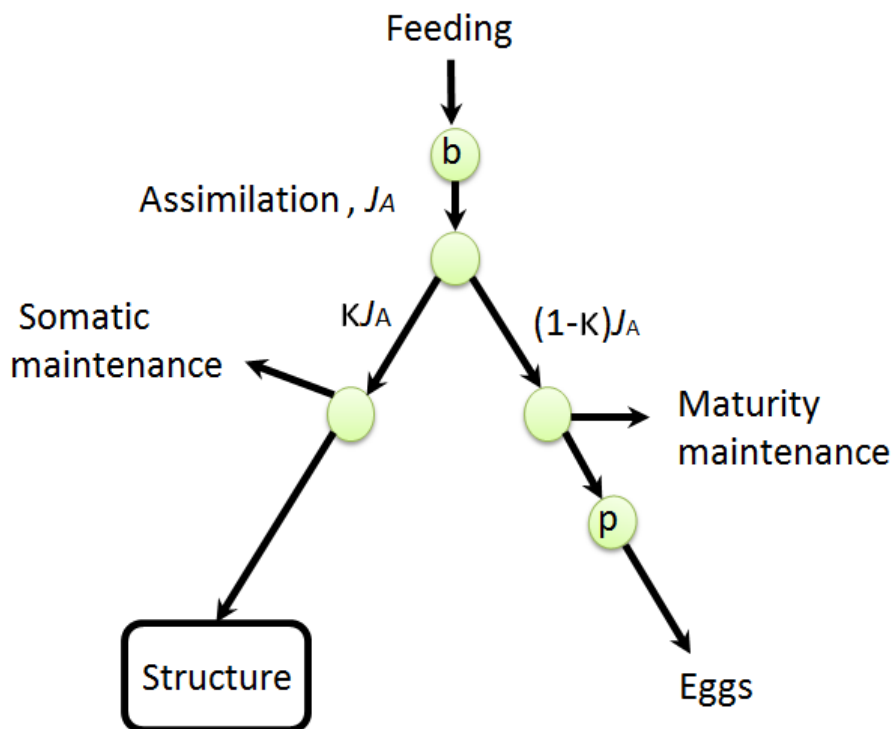


Figure 6.1: Schematic diagram of the DEBkiss model we applied in the study. The flow of mass indicated with arrows. The assimilated flux, J_A , is distributed in a fixed fraction (κ) between somatic growth/maintenance and maturity maintenance/reproduction. The circles containing "b" and "p" indicate maturity switches for birth and puberty, where feeding and allocation to reproduction begin respectively.

cess is directly related to the concentration of the toxicant inside the organism. Thus, the DEBkiss model can be extended to model the effects of toxicants by including two models: a model that links ex-

ternal concentrations to internal concentrations (toxicokinetics, TK), and a model that links internal concentrations to effects on one or more of the parameters of the energy budget (toxicodynamics, TD). A useful modeling framework for TK and TD in this context was provided by Jager et al. [18] in their supporting information.

Toxicokinetics model

The TK model departs from the assumption that we can describe the time course of Cd inside *F. candida* using a first-order one compartment model, accounting for changes in size of the compartment. The core assumption for this model is that the uptake rate is proportional to the environmental concentration, and the elimination rate is proportional to the internal concentration. The first-order one compartment TK model can be used for different internal concentration measures (see [18] for the detailed description of the different internal concentration measures and the corresponding TK model equations). In this study, we used the scaled internal concentration; because, we do not have body residue data.

$$\frac{d}{dt}c_V = k_e^* \frac{L}{L_m} (c_d - c_V) \quad (6.1)$$

where c_V is the scaled internal concentration of Cd, c_d is the Cd concentration in the food and k_e^* is the reference elimination rate

constant at maximum size, L_m .

Toxicodynamics (TD) model

The TD model needs to link the scaled internal concentration, c_V to effects on one or more of the DEBkiss model parameters. We assume a linear relationship with threshold between the internal concentration and the degree of stress (s) on a model parameter:

$$s = \frac{1}{c_T} \max(0, c_V - c_0) \quad (6.2)$$

where s is a dimensionless indicator of the degree of stress on a model parameter, c_T is called the 'tolerance' concentration, and c_0 is the threshold concentration for effects. The TD parameters c_0 and c_T are estimated by fitting the model to the toxicity data.

6.2.5 Modeling the effect of temperature

In a DEB context, temperature affects all metabolic rates in the same way, and its effect can be included by multiplying all rate parameters with a correction factor from the Arrhenius equation [23]. In our study, we assumed that temperature affects the specific rates for assimilation and maintenance, as well as the time needed for the juveniles to feed at the maximum rate (T_f , see previous chapter). The parameter values at 20 °C ($T_{ref} = 293$ K) were used as reference

values, and corrected for any temperature, T (in Kelvin) by:

$$F_T = e^{\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right)} \quad (6.3)$$

where T_A is the Arrhenius temperature in Kelvin. The value is species specific and we estimated it from the growth and reproduction data collected at three different temperatures.

6.2.6 Parameter estimation

The set of parameters that needs to be estimated can be categorized into three main groups: the basic DEBkiss parameters, the TK/TD parameters, and the Arrhenius temperature, T_A , a parameter which describes the effect of temperature. The DEBkiss model parameters and the Arrhenius temperature, T_A for *F. candida* have already been estimated in a previous study (Chapter 5 of the thesis). Thus, in this study we only estimated the TK/TD model parameters for the effect of Cd using the data obtained from the toxicity experiments.

The TK/TD model involves three parameters, namely, the reference elimination rate, k_e^* , the no effect concentration c_o and the tolerance concentration, c_T (Table 6.1). We estimated these parameters using the maximum likelihood optimization (assuming independent normal distributions for the residuals after square root-transformation of the growth and reproduction data), and derived the confidence intervals

by profiling the likelihood (detailed description of the method can be found in [24]). All calculations were performed with a program code developed in MATLAB (Version 8.0.0.783, R2012b).

6.2.7 Model simulations

Predicting individual-level effects of Cd and temperature

We used the parameterized model to predict the effect of Cd on growth and reproduction of *F. candida*. We predicted EC_x values (the concentration of Cd at which growth and reproduction were reduced to $x\%$ of that of the control) at different exposure times and temperature conditions. We used this information to analyze how temperature and exposure time influences these individual-level effect indices.

Predicting population-level effects of Cd and temperature

We linked the DEBkiss model to the Euler-Lotka equation to calculate the intrinsic population growth rate, r :

$$\int_{a_b}^{a_d} S(a)R(a)e^{-ra}da = 1 \quad (6.4)$$

Where a is age (in day, starting with the fresh egg at $a = 0$); $S(a)$ is the probability for an individual to survive up to age a ; $R(a)$ represents the reproduction rate of an individual of age a , (# eggs per day); r the intrinsic population growth rate, (day^{-1}). a_b is the age at

birth (hatching time of eggs) and a_d is the time when we stopped the experiment after 85 days post hatching (the last date of observation for egg production).

Except for the highest study temperature, the Cd concentration we studied influences only growth and reproduction of *F. candida*. Thus, we assumed $S(a) = 1$ for both 15 and 20 °Cs temperature conditions.

6.3 Results

6.3.1 Parameter estimates, model fit and mode of action

Due to the low reproduction and higher mortality rate observed at 25 °C, it was not possible to fit the model and make reliable model predictions at this temperature. There was no reproduction at 500, 1500 and 2000 mg/kg. After 42 days of exposure less than 35% of individuals survived from the two highest Cd concentrations. Thus, only data obtained from 15 and 20 °C were used for the parameterization of the model and further analysis will be based on results from these two temperatures.

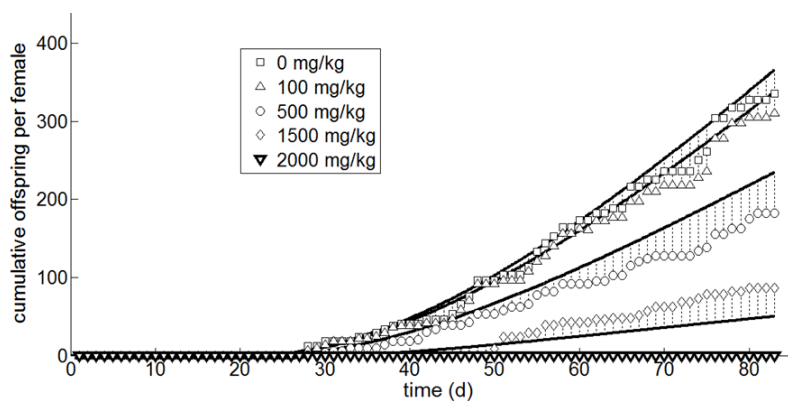
Table 6.1 shows the estimates for the TK/TD parameters used in our model, while Figures 6.2(a), 6.2(b), 6.3(a) and 6.3(b) show the model fit to the data. We fitted the model by considering that Cd decreases the assimilation efficiency of *F. candida*, which leads to effects on both growth and reproduction. An increase in somatic maintenance or an

increase in the costs for structure can have similar effects, but our data are best described by a decreased assimilation efficiency (the final fit in the two MoA are shown the Appendix part). Jager et al. [27] also selected the same mode of action (MoA) for the effect of Cd on *F. candida*. The reference elimination rate, k_e^* , is influenced by physiological rates, which are temperature dependent. Thus, we estimated a temperature dependent k_e^* . As shown in Table 6.1, we also assumed that temperature influences c_T , hence the assumption resulted the best fit (the fit by considering temperature independent c_T value is shown in the Appendix part).

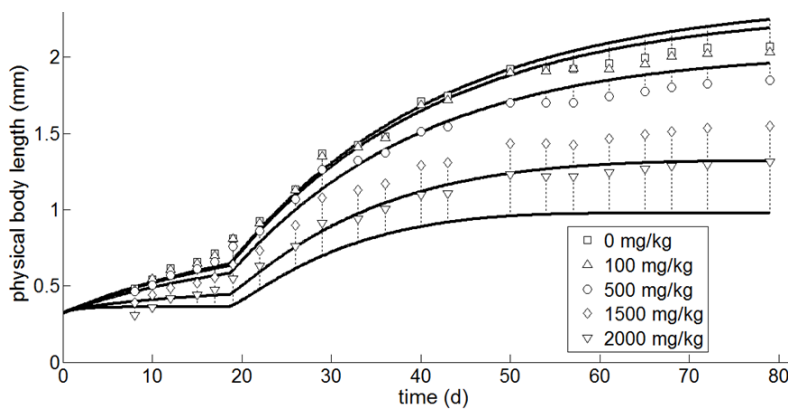
As shown in 6.2 and 6.3, the growth and reproduction data of the control and four Cd concentrations at the two temperature conditions are well predicted by the model.

Table 6.1: Model parameters and estimated values, using maximum likelihood optimization. Values in brackets are the 95% confidence intervals, CIs, derived using profile likelihoods.

Parameter		Value		Description
		T = 15 °C	T = 20 °C	
k_e^*	day ⁻¹	0.117 (0.112–0.122)	0.194 (0.209)	Elimination rate constant at maximum size
c_T	mg/kg	3358 (3350–3376)	5321 (5277–5391)	Tolerance concentration
c_o	mg/kg	$3.76 \cdot 10^{-5}$ (0–3.178)	$3.76 \cdot 10^{-5}$ (3.178)	Threshold concentration

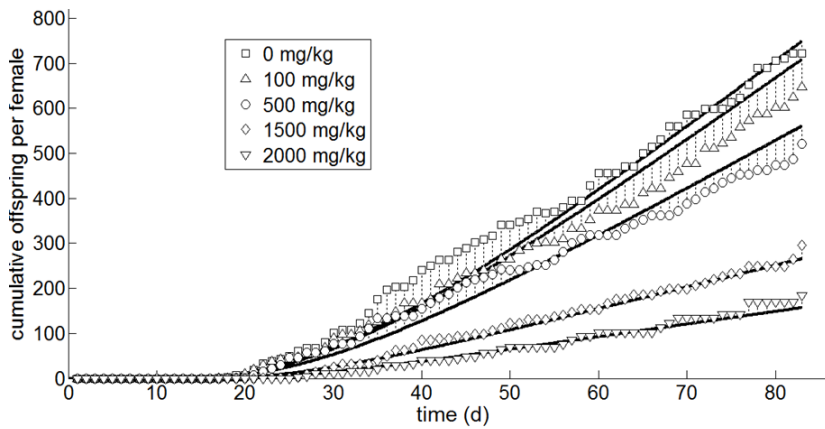


(a)

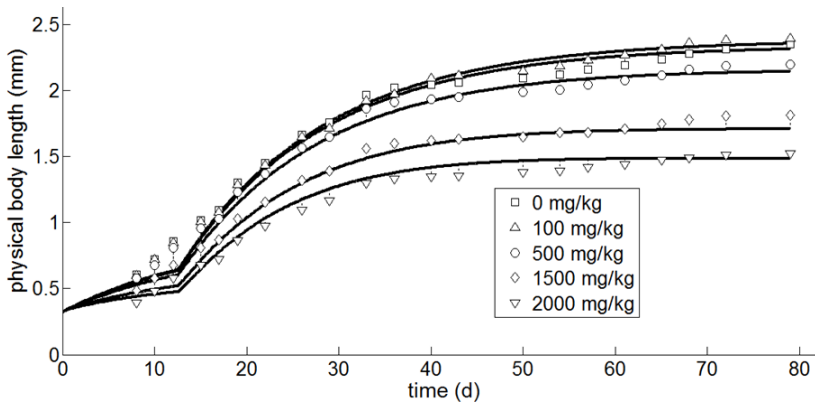


(b)

Figure 6.2: The model fitted to the growth and cumulative reproduction data obtained from the toxicity experiment at the 15 °C. The symbols are the mean values observed at the different Cd concentration levels (displayed in the legends). The lines correspond to the model predictions.



(a)



(b)

Figure 6.3: The model fitted to the growth and cumulative reproduction data obtained from the toxicity experiment at 20 °C. The symbols are the mean values observed at the different Cd concentration levels (displayed in the legends). The lines correspond to the model predictions.

6.3.2 Effects on physical body growth

Figure 6.4 shows the $EC_{25_{growth}}$ as function of time at 15 and 20 °C. It appeared that $EC_{25_{growth}}$ values decrease with time and reach a steady state after some time. It is also clearly shown that the EC_{25} values at 20 °C are relatively higher than the corresponding $EC_{25_{growth}}$ at 15 °C. As shown in the figure, at 20 °C the steady state $EC_{25_{growth}}$ is about 1.5 times higher than that of at 15 °C. This suggests that at 15 °C, growth in *F. candida* is generally more affected by Cd than that at 20 °C. This might be because of the fact that EC_x is determined by many factors, such as k_e^* and c_T . But also because the physiological rates, like body growth rate and reproduction rate are temperature dependent.

6.3.3 Effect on cumulative reproduction

Unlike the growth; the EC_x for cumulative reproduction increases with time and reaches a steady state condition. Similar to $EC_{x_{growth}}$, temperature influences the $EC_{x_{reproduction}}$. For instance, as depicted in Figure 6.5 at 20 °C, the steady state $EC_{50_{reproduction}}$ is about 1.61 times higher than the $EC_{50_{reproduction}}$ at 15 °C. This suggests that at 15 °C, reproduction in *F. candida* is generally more affected by Cd than that at 20 °C. It is also clear that Cd has larger effects on reproduction than on growth.

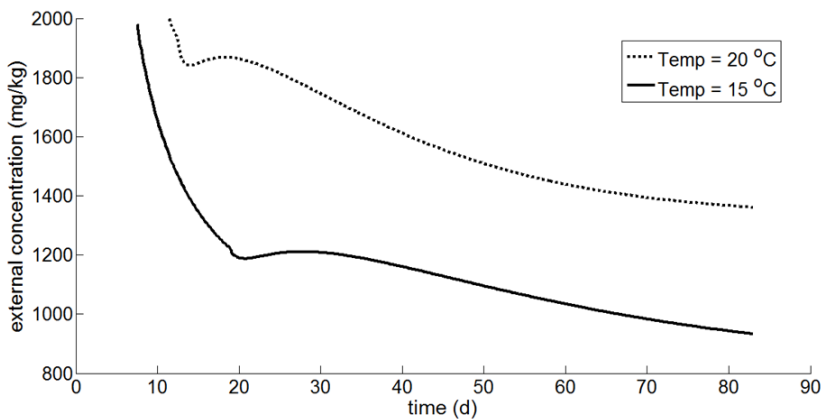


Figure 6.4: $EC_{25_{growth}}$ values (the concentration of Cd at which growth was reduced to 25% of that of the control) at 15 °C (solid line) and 20 °C (broken line).

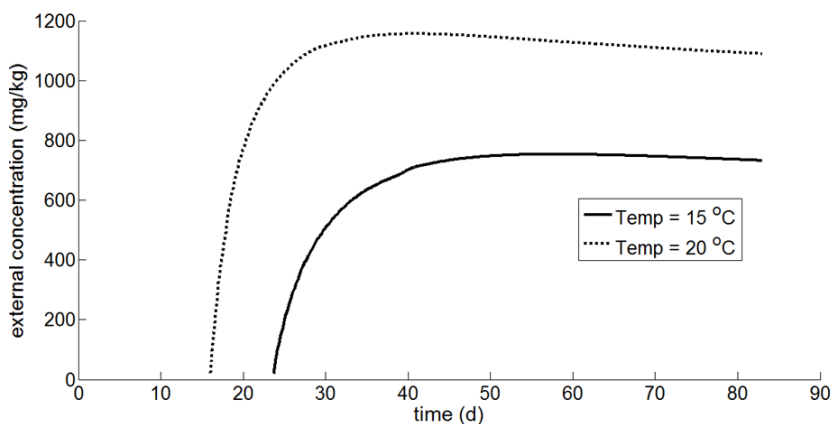


Figure 6.5: $EC_{50_{reproduction}}$ values (the concentration of Cd at which cumulative reproduction was reduced to 50% of that of the control) at 15 °C (solid line) 20 °C (broken line).

6.3.4 Effect on population growth rate

Using the DEBkiss model and the Arrhenius relationship Equation 6.3, we can easily estimate body size and reproduction rate at different Cd concentrations at both 15 °C and 20 °C. As shown in Figure 6.6, for both temperature conditions, the predicted population growth rate decreases with increasing Cd concentration monotonically. Except at highest Cd concentration and 15 °C condition, the predicted intrinsic population growth rate remained positive.

We further performed an analysis to identify whether temperature

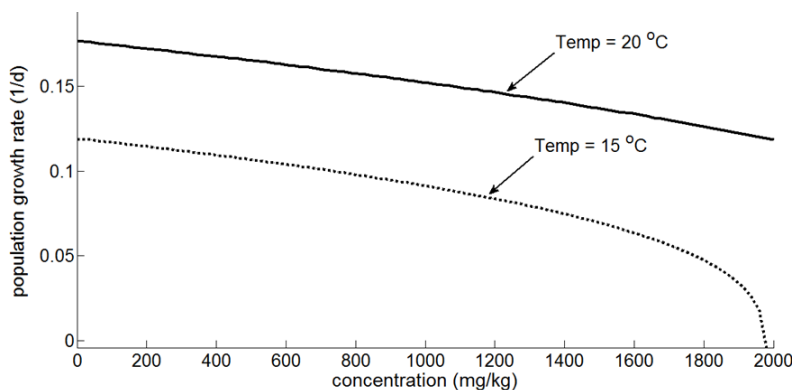


Figure 6.6: The predicted population growth rate as function of Cd concentration at 15 °C (broken line) and 20 °C (solid line).

influences the population-level effect of Cd, and whether the two stressors interact. We calculated the relative population growth rates as function of Cd concentration for both temperature conditions (shown

in Figure 6.7) as the ratio between the population growth rate of the exposed population to the control population. At 15 °C, the relative population growth revealed a sharper decline than at 20 °C. Thus, for the same environmental concentration of 1600 mg/kg Cd in food, the population growth rate is reduced by 20% for the population living at 20 °C and by 42% for population living at 15 °C. This implies an interaction between Cd and temperature, at least for the temperature range considered in this study.

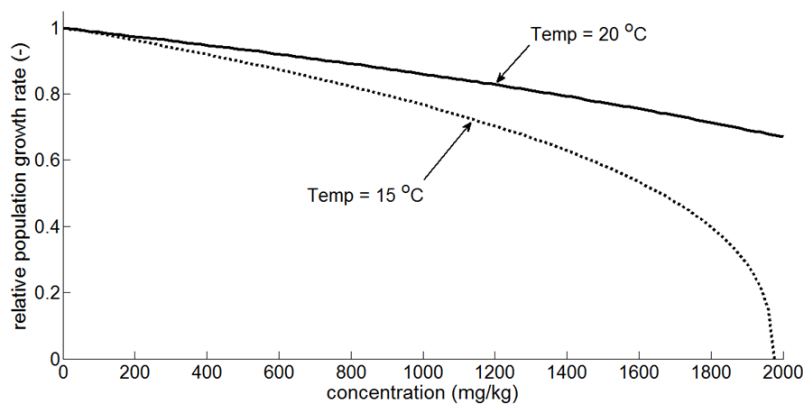


Figure 6.7: The combined effects of Cd and temperature on the population growth rate of *F. candida*. The predicted relative population growth rate as function of Cd concentration at 15 °C (broken line) and 20 °C (solid line).

6.4 Conclusion

Temperature is a key environmental factor that influences both the physiology of animals and physico-chemical processes in the natural environment. Thus, interpretations of effects observed in laboratory, which are usually standardized to a constant temperature condition of 20 °C, to different temperature conditions requires consideration of the influence of temperature variation. Similarly, extrapolation of effects observed on individuals to populations living at different temperature conditions might lead to erroneous conclusion. In this study, we applied the simplified DEB model (DEBkiss) to explore the combined effects of Cd and temperature on the vital rates of *F. candida*. By coupling the DEBkiss model with the Euler-Lotka equation we were able to extrapolate the combined effects of the two stressors to the population-level. The model provides a mechanistic understanding of the effects of the two stressors at individual-level and helps to make an educated extrapolation. Our results show that the combined effects of Cd and temperature on the life history of *F. candida* are predictable using the DEBkiss model, at least for the temperature range between 15 and 20 °C. We also showed that the effect of Cd on the population growth rate of *F. candida* is influenced by temperature. Thus, the model framework presented in this study is a useful tool to improve the current chemical risk assessment process as it allows

extrapolation of effects observed in the laboratory to populations in the field, where temperature is temporally variable and differs from laboratory conditions. Implementing an Arrhenius type function for the effect of temperature on the TK/TD parameters improves the model's ability for extrapolating effects from one temperature condition to another.

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Appendix *Preliminary results of the model fit*

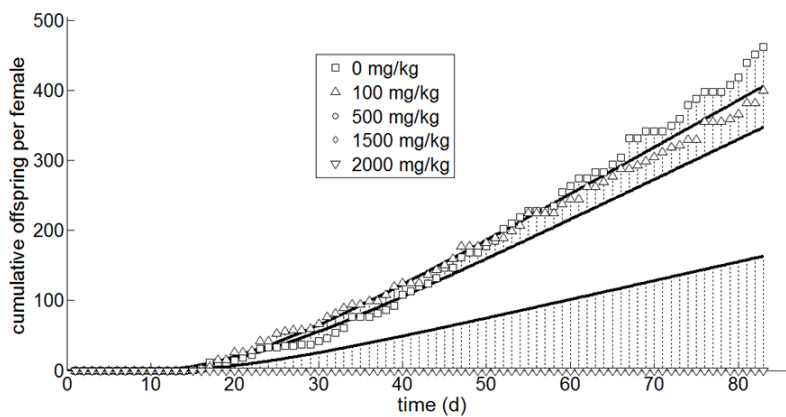
Fitting the model to 25 °C data

As shown in Figures 6.8(a) and 6.8(b) the model was able to capture only the data for the control and the lowest Cd exposure.

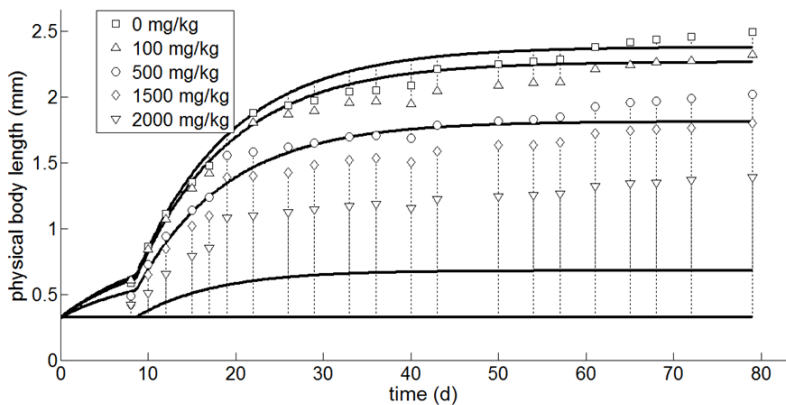
Fitting the model for different toxicant mode of actions (TMoA)

As was mentioned in the main text, ‘Increase in somatic maintenance’ and ‘Increase in costs for structure’ MoAs also affect reproduction and growth simultaneously. However, as shown in the following figures, these assumed MoA as well as the selected MoA, assimilation, could not capture the trend of the data fully.

Increase in somatic maintenance



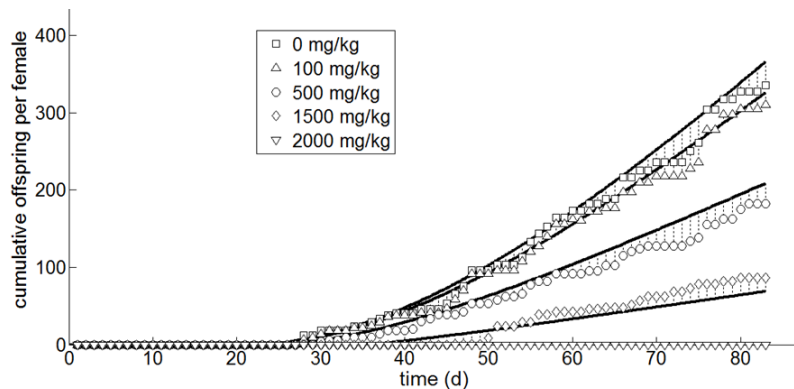
(a)



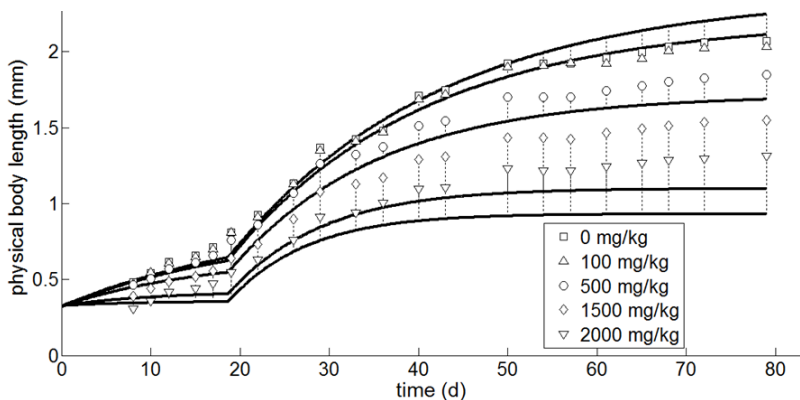
(b)

Figure 6.8: The model fitted to 25 °C data. Cumulative reproduction (top) and physical body length (bottom).

Increase in costs for structure



(a)

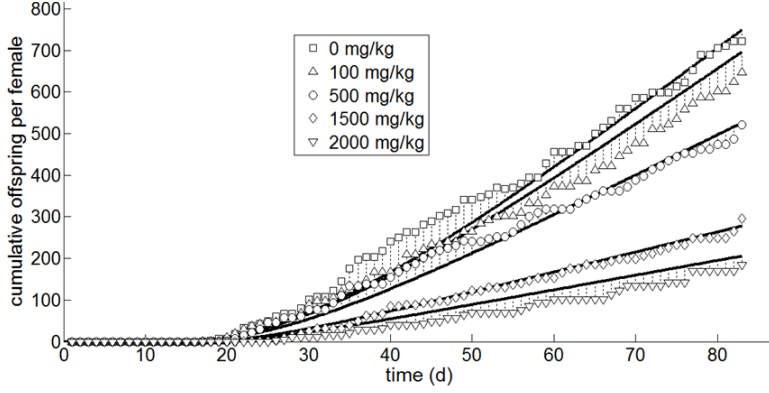


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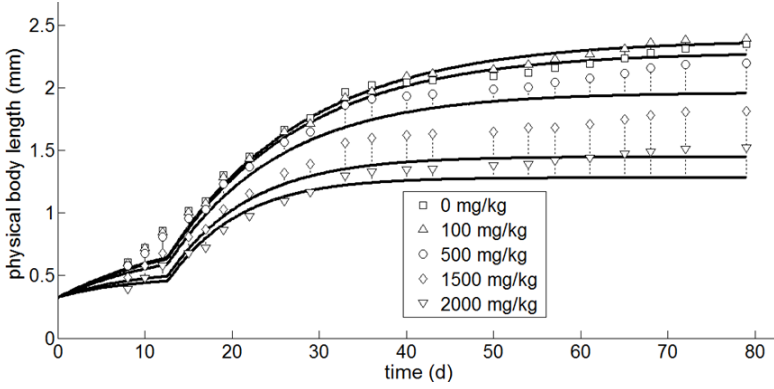
Figure 6.9: The model fitted to 15 °C data. Cumulative reproduction (top) and physical body length (bottom).

Fitting the model assuming temperature independent TD parameters

The model was initially fitted by assuming that temperature influences only the reference elimination rate, k_e^* . As shown in Figures



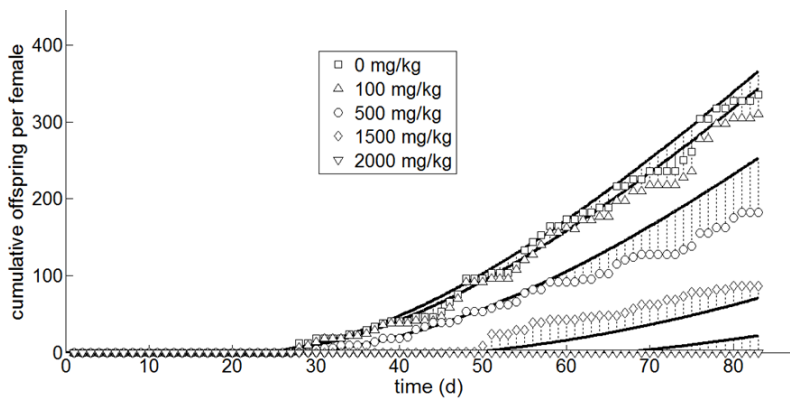
(a)



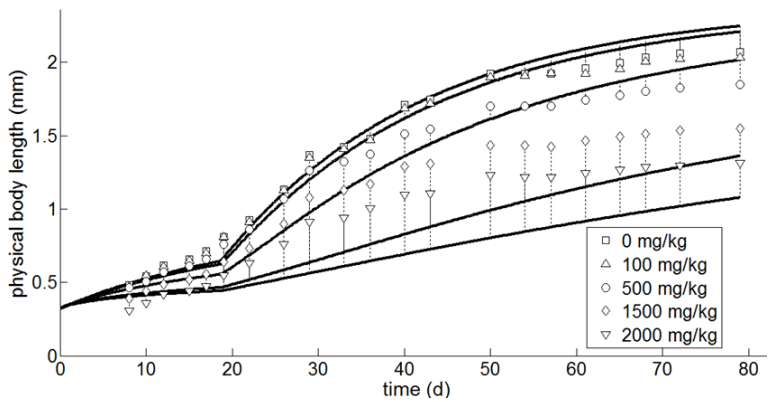
(b)

Figure 6.10: The model fitted to 20 °C data. Cumulative reproduction (top) and physical body length (bottom).

6.13(b), 6.14(a), and 6.14(b), the assumption gives a very reasonable fit to all data apart from reproduction at 15 °C (6.13(a)). Thus, we assumed a temperature dependent k_e^* and c_T , which resulted a better fit (see Figures 6.2(a), 6.2(b), 6.3(a) and 6.3(b))

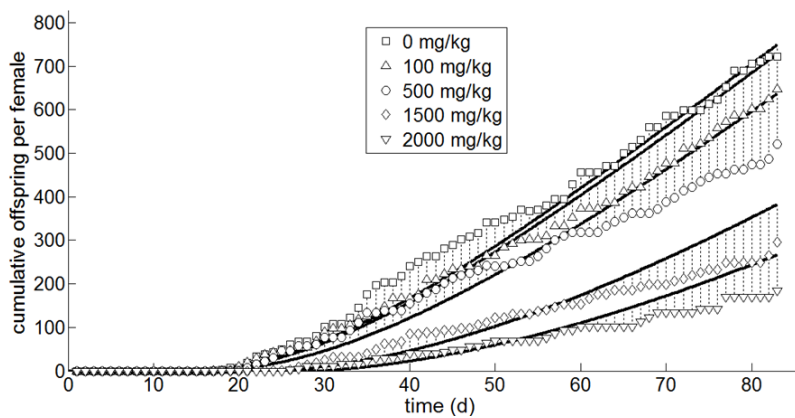


(a)

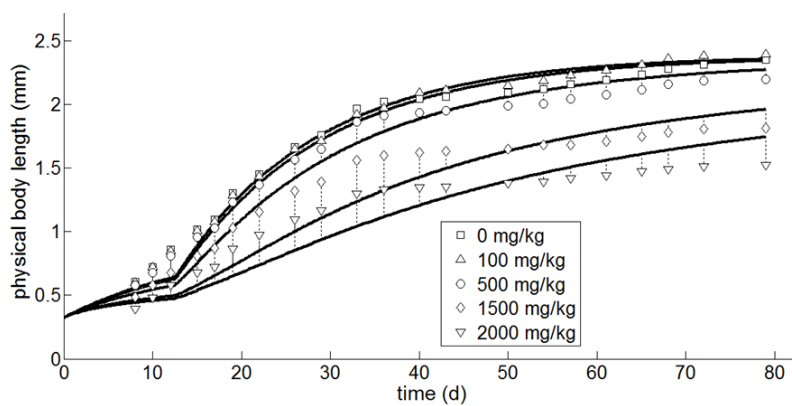


(b)

Figure 6.11: The model fitted to 15 °C data. Cumulative reproduction (top) and physical body length (bottom).

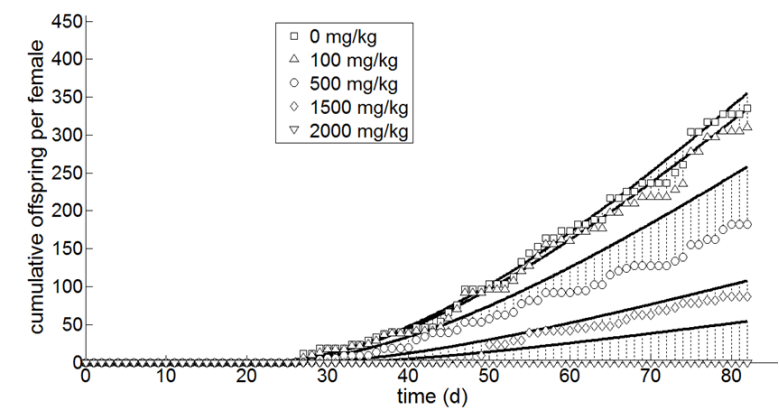


(a)

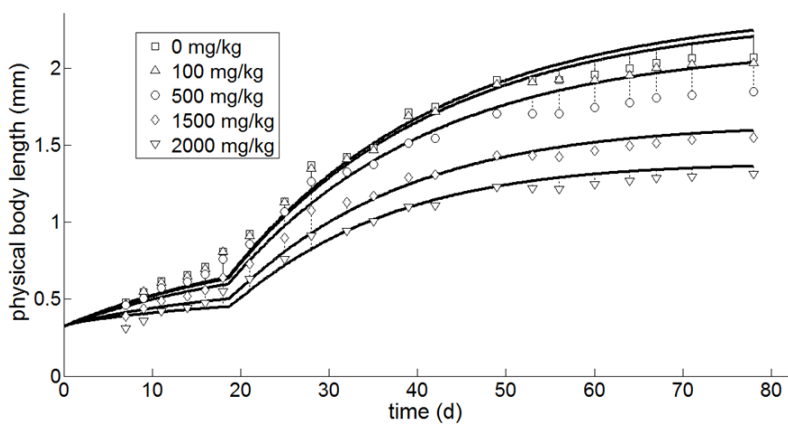


(b)

Figure 6.12: The model fitted to 20 °C data. Cumulative reproduction (top) and physical body length (bottom).

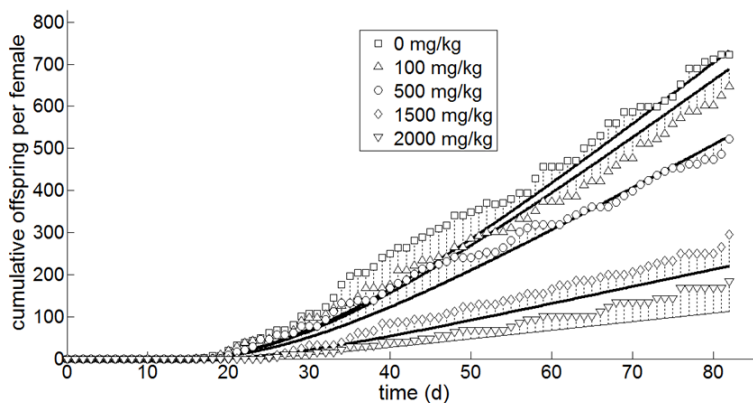


(a)

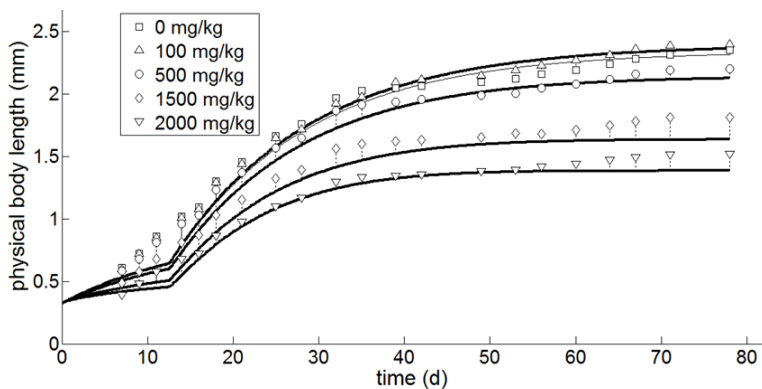


(b)

Figure 6.13: The model fitted to 15 °C data. Cumulative reproduction (top) and physical body length (bottom).



(a)



(b)

Figure 6.14: The model fitted to 20 °C data. Cumulative reproduction (top) and physical body length (bottom).

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Chapter 7

General Discussion

7.1 Overview

The research described in this thesis was conducted within the framework of the project “Mechanistic effect models for ecological risk assessment of chemicals: CREAM”, <http://cream-itn.eu>. The project aimed to develop different mechanistic effect models (MEMs) for risk assessment of chemicals, for different species and different purposes. Among others, developing population models for the extrapolation of individual-level effects observed in standard bioassays to the population level was one of the objectives of the project.

The aim of this thesis was to describe and model the effects of biotic and abiotic factors on toxicity of chemicals at the individual and population level, and develop population models that can predict and extrapolate these combined effects from the individual level to populations. More specifically, I tested and developed different extrapolation techniques and types of generic population models and investigated their applicability using case studies. As a start, in Chapter 2, I presented a matrix population model based mathematical analysis that can help in designing better short term toxicity tests which, by taking

into account species-specific life history and sensitivity of particular life-history traits to toxicants, allow for more robust estimation of population-level effects. Subsequently, I developed a generic structured population model for the extrapolation of the combined effects of toxicants and ecological factors to the population level. Initially, I focused on a model that can be developed and parameterized using data gathered according to current toxicity test protocols and easily available environmental data. A more generic and mechanistic effect model, which requires more toxicity data, was developed by integrating an energy budget model with an Euler-Lotka equation.

The theoretical background and the rationale of these models and model application case studies are summarized in the outline of the thesis in Chapter 1, and covered in detail in Chapters 2-6. In this chapter, I will summarize and discuss the results of the study, emphasizing the main features of the models in light of answering the research questions posed in Chapter 1, and the potential applicability of the models as a tool in the ERA process. I will also reflect on some of the questions that came out of the study and suggest possible future work and directions for improvements.

7.2 Linking individual-level effects and population-level consequences: A central theme of ecotoxicology

Ecological risk assessment (ERA) serves as an important decision making tool in the protection of the environment and human health from adverse effects of chemicals. As its name indicates, ERA should involve the complexity of natural ecosystems and effects should be expressed in terms of ecologically relevant end points. Thus, ecological risk management decisions are essentially aimed at ensuring protection at higher levels of biological organization, e.g., populations or entire ecosystems and thus risk assessment at these higher levels biological organizations is emphasized in major ERA directives, guidelines and policy documents [1,2]. Particularly, there is a growing interest to assess ecological impacts of chemicals at the population level [3–5]. Unfortunately, assessing effects of chemicals at the population level is impractical, because effects depend not only on exposure and toxicity, but also on important ecological factors that are impossible to fully address empirically [6]. Further more, population level effect assessment for some species is ethically unjustifiable, or even prohibited [7]. Thus, effects of chemicals are usually assessed from results of laboratory toxicological experiments performed on individuals of selected species. The effects are determined by following individual end-points, such as changes in body size, reproduction rate

and short-term survival. These end-points are easy to measure and the data are inexpensive to produce, hence the tests are standardized and widely used for chemical risk assessments. Linking these observations to effects at the population level is therefore the central goal of ecotoxicology [8], and ecotoxicologists are increasingly turning their attention to the relationships between effects of chemicals on individual life-history traits and at the population level [9]. Effects measured at the level of individuals can be extrapolated to expected population-level responses using different mathematical and simulation models [10].

7.3 Models to extrapolate individual-level effects to a population

Population models for the extrapolation of individual-level effects to the population level help to understand how changes in vital rates manifest themselves in population consequences (i.e., mechanistic understanding). Several modelling approaches have been developed and used for the extrapolation of effects measured at the level of individuals to expected population-level responses. Barnthouse et al. [4] classified population models into five general categories: unstructured (scalar), biologically structured, individual based, metapopulation, and spatially explicit. This classification is based on the level of bi-

ological and spatial aggregation reflected in the models as they are typically constructed and used, and the classes are not mutually exclusive [4]. Given the range of options for linking individual-level

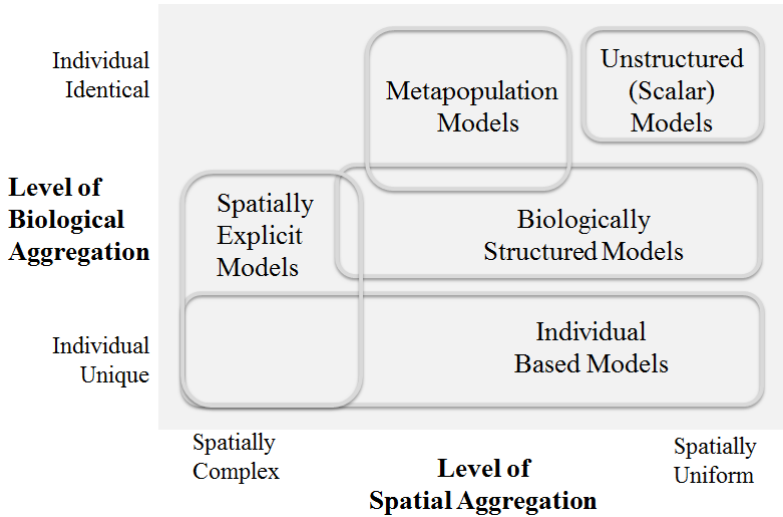


Figure 7.1: Taxonomy of population models for population-level ecological risk assessment. These models range from extremely simple to highly complex. It should be noted that these classes are not mutually exclusive; for example, one can create a spatially explicit individual based model. Rather, individual model formulations fall along continua of biological and spatial aggregation instead of into discrete classes. This taxonomy is meant to provide a general idea of the potential applications and issues associated with broad model classes (re-used from [4] with permission).

effects to population responses, which model should be used? That choice is situation-dependent and should reflect a number of considerations, including the assessment endpoint and how risk estimates will

be used in the decision process, the model's intended use, the biology of the assessed species and the availability of data describing it, the resources available for performing the assessment, and so on. Additionally, the informational requirements of the selected model must be satisfied [11].

In this study, I applied biologically structured population models: matrix population models and the Euler-Lotka equation. These two classes of models specifically include demographic characteristics of the organism and they tend to reflect biological realism of the species to be modelled [4]. These models are popular and widely used for extrapolation of individual-level effects to population responses of toxic chemicals. Both models estimate the population multiplication factor (λ) and the population growth rate (r), an end-point which is viewed as a fitness measure for the population (i.e., a measure of the ability to maintain a healthy population growth). Forbes and Calow [9, 12], and Barnthouse et al. [4] discussed the suitability of using population growth rate as a measure of population-level toxicant effects.

In Chapter 2, I applied an age-classified matrix model, while Chapter 3 and 4 are based on a stage-based matrix model. On the other hand, in Chapters 5 and 6, I applied the Euler-Lotka equation. The selection of the models is based on two important factors: the research questions we wanted to answer and data availability. For example, in

Chapter 2, I constructed an age-based matrix model using full life-cycle data on the effects of cadmium (Cd) and imidacloprid on reproduction and survival of aphids (*Acyrtosiphon pisum* Harris) to estimate the population growth rate at different exposure levels. Further, I implemented a demographic decomposition analysis and elasticity analysis to point out how population models can help in designing better short-term ecotoxicological tests (discussed in Section 7.4). Most standard toxicity tests are performed only on part of the life cycle of organisms. Thus, in Chapter 4, I implemented a stage-based matrix population model for the extrapolation of individual-level effects by considering two important ecological factors: seasonal temperature fluctuation and density dependence. In Chapters 5 and 6, I linked DEB based individual-level effect model with the Euler-Lotka equation to extrapolate individual life history data to population level. Linking the Euler-Lotka equation with DEB based individual-level effects model is straightforward. In contrast, a matrix model would require a translation of DEB results to the ‘vital rates’ in the matrix, and a discretisation of the life-cycle of the organism; a procedure that can be done in many different ways [13]. This makes the Euler-Lotka equation more suitable than the matrix model for the extrapolation of individual-level mechanistic effect models, which are derived from DEB theory. On the other hand, matrix models are useful to analyse

the sensitivity of the population growth rate to quantitative changes in vital rates. Life table response experiments (LTRE) in general, and sensitivity analysis in particular, are suitable tools to explore how toxicant-caused changes in individual life-history traits influence the sensitivity of the population growth rate. The very detailed life history data required for parameterization makes the Euler–Lotka equation unsuitable for extrapolations based on data originating from standard toxicity tests.

7.4 To what extent does the individual-level sensitivity influence population-level responses?

One of the fundamental problems confronting population-level ecotoxicology is how to infer the consequences for a population from toxicant effects observed in the individuals [15]. This is because currently ecotoxicological tests are usually used to determine a dose–effect relationship for a given chemical for a certain endpoint at a fixed time point. Although these short-term ecotoxicological studies are practical from an economic point of view, they cannot provide us sufficient information to estimate population-level end-points like population growth rates r and λ . Thus, for such individual-level responses to be useful endpoints, it is necessary that they adequately and consistently reflect the impacts of chemicals on populations [12]. In this respect,

Forbes and Calow [12] raised two important issues of concern. The first is whether individual responses in survival, fecundity or growth and development are more or less sensitive to chemical impacts than effects on population growth rate. The second is whether there is consistency in the relationship between changes in the individual traits and changes in population growth rate, such that it is possible to identify those traits that are the best predictors of effects on population growth rate. In Chapter 2 of the thesis I tried to answer these questions. I showed that by investigating the most important vital rates, with the largest contributions to reduction of the population multiplication factor $\Delta\lambda$, it is possible to link the individual-level effects to the population-level impacts. The approach presented in this chapter of the thesis provides the mathematical details for how to use Life Table Response Experiment (LTRE) decomposition analysis to determine the optimal approach for designing ecotoxicological assessments. The method was applied to analyze the age-specific toxic effects of cadmium (Cd) and imidacloprid on reproduction and survival of aphids (*Acyrtosiphon pisum* Harris) and compared the respective contribution of effects on each vital rate to the differences in the population multiplication factor between the treated groups and the control group ($\Delta\lambda$).

Age-classified matrix models were constructed from daily life table

data to estimate λ at each treatment level. The relative contribution of changes in each life-history trait to the difference in population multiplication factor between the control and a treatment ($\Delta\lambda$) were examined using the LTRE decomposition analysis presented in the thesis. The results showed that the population-level interpretation of the individual-level experimental results requires information on the effect of the toxicant on the life stage under study and the demographic sensitivity of λ to changes in particular life stages.

The analysis result showed that, despite of different results expected from the two toxicants, the elasticity values were very similar. The elasticity of λ with respect to survival was highest in the first age class, and that with respect to fertility was highest in the second age class. The demographic decomposition analysis examined how changes in life-history traits contributed to differences in λ between control and treated populations ($\Delta\lambda$). This indicated that the most important contributors to $\Delta\lambda$ were the differences in survival (resulting from both demographic sensitivity and toxicity) in the 1st and the 2nd age classes of aphids and differences in fertility in the 3rd and the 4th age classes. Additionally, the toxicants acted differently. Cadmium reduced λ by impairing fertility at the 3rd age class and reducing survivorship from the 2nd to the 3rd age class. Imidacloprid mostly reduced survivorship at the first and second age classes.

The elasticity and decomposition analyses showed different results, because these methods addressed different questions about the interaction of organism life history and sensitivity to toxicants. I suggest the LTRE may be useful for designing individual-level ecotoxicological experiments that account for both the effects of the toxicant and the demographic sensitivity of the organism.

The approach I presented in this LTRE study can help to identify and select the most relevant life stages for individual-level toxicity tests. For ecological risk assessment, such tests should be designed taking into account the overall contributions of toxicity in particular life stages to population dynamics, which result from sensitivity of λ to changes in particular vital rates and the responsiveness of these vital rates to particular chemicals. I showed that these contributions are different for different chemicals, which indicates that the design of bioassays should be chemical-specific. The main disadvantage of this approach is that development of life tables is time consuming and expensive. It might be possible, however, to run just one full LTRE per species for each major class of chemicals with the same mode of action, and to build on these grounds test designs specific for a species and for each class of chemicals. Particularly, if the current standard toxicity tests continue to be the main information source for Ecological Risk Assessment (ERA) of chemicals, the method presented in

this LTRE study may overcome its disadvantages.

The major result of this chapter of the thesis is the need for revising the current standard toxicity test design. In general, the prevailing view in most of the current standard laboratory tests is to focus on effects at what are considered to be the most sensitive stages of the life cycle. However as it was presented in my study findings, this sensitive life stage-based toxicity tests has shortcomings. Kamminga et al. [16] also showed how the direct extrapolation of sensitive life stage-based tests to population-level response might lead to erroneous conclusions.

7.5 To what extent do key ecological factors influence population-level effects of toxic chemicals?

It is well recognized that natural stressors as well as key ecological factors influence the effects of toxic chemicals on individuals and higher levels of biological organization (e.g. [17–20]). With regard to ecological risk assessment of toxic chemicals it is important to know how and to what extent these key ecological factors can modify effects of toxicants at different levels of biological organization. This is because information on the ecotoxicity for population-level risk assessment is still derived from laboratory bioassays, which are conducted under constant and, presumably, favorable conditions. However, in their

natural environment organisms rarely experience optimal conditions, and these conditions are rarely constant. On the contrary, organisms are forced to cope with either suboptimal or severe environmental stress for most of their life spans [18,19]. These added environmental factors may or may not alter the effects of chemical contaminants in comparison to laboratory tests performed under controlled and optimal conditions. Therefore, understanding and including ecological factors that can modify effects of toxicants at individual and population levels is a key step in developing tools for extrapolating the results of laboratory toxicity tests to populations living in natural environments. In this thesis, I add to the general knowledge of these aspects of population-level chemical risk assessment. In Chapter 4, I implemented a generic stochastic density-dependent matrix (SDDM) model for the extrapolation of individual-level effects to the population level in natural environments. In the model, I considered the influence of seasonal environmental variability and density-dependence on the population-level effects of toxicants. In developing the model, I considered a suite of generic models that can be parameterized with easily available data, particularly toxicity data from current standard individual-level tests. Figure 7.2 shows the conceptual framework of the SDDM model. As shown in the figure, the different elements of the model are derived from realistic ecological assumptions.

Using an age/stage structured matrix model framework, I included the effects of toxicants, environmental variability and density-dependence by developing separate sub-models. In developing the sub-models, I applied and used well-proven and tractable mathematical formulations for the functional representation of the SDDM model, which makes it robust. I performed five main steps: formulating the projection matrix, modeling individual-level effects of toxicants, estimating environmental stochasticity, modeling density-dependence and estimating overall population-level effects. An important step worth mentioning here is the estimation of the environmental stochasticity. I started with exploring existing methods for estimating environmental stochasticity using matrix population methods. In general, environmental stochasticity can be incorporated into matrix models by either selecting whole observed deterministic matrices at random at each time step of a simulation or by selecting the vital rates at random from some statistical distribution [21]. Based on these two generalized approaches, three methods are currently used to formulate and analyze stochastic matrix models. Fieberg and Ellner [22] technically categorized these three methods as "random transition matrix" (RTM); "parametric matrix method" (PMM) and "small fluctuations approximation" (SFA); the analytical method which was proposed by Tuljapurkar [23]. Unfortunately, the direct application

of these methods for including environmental stochasticity in matrix models is difficult for organisms used for standard toxicity tests; they are typically developed for long-lived animals. These methods require the derivation of environment-dependent matrices together with their frequency distributions, which are usually calculated based on year-to-year changes in vital rates. However, for species with a short lifespan, collecting environment-dependent demographic data from the field is often not feasible from a technical and/or economical point of view. In Chapter 3, I developed a methodology, which combines easily available and/or measurable laboratory-based demographic data with actual field-measured climate data, to estimate environmental stochasticity for animals with a short life span. Using the model, I was able to explore the effect of seasonal temperature variability on *Folsomia candida* populations. Using the model, I estimated the stochastic population growth rate of *F. candida* and the degree of environmental variability arising from the seasonal temperature fluctuation for a specific study area I considered. Using the theoretical approach developed in Chapter 3, and a density-dependent function derived from published data, in Chapter 4, I illustrated the potential applicability of the SDDM model to extrapolate the combined effects of Cd, density-dependence and seasonal temperature fluctuation on *F. candida* population. The matrix population model presented in the

case study is a three stage model, which involves the three important life stages of the organism, the embryonic, juvenile and adult stages. I used this three stage matrix model to extrapolate the individual-level endpoints to the population-level. In Chapter 4, I showed that extrapolating individual-level effects of Cd to a higher level of biological organization without considering environmental variability underestimates effects of chemicals on the population growth rate. The simulation results from the model also showed that chemicals can reduce the population size at its carrying capacity. The latter phenomenon arises from different effects of toxicants on different age/stage classes combined with natural demographic processes. One important and useful population-level endpoint that can combine all these effects (i.e., environmental variability, chemical-specific toxicity, and density-dependence) is the mean time to extinction. The model simulation results showed that the mean time to extinction of the *F. candida* population exposed to 1000 mg Cd/kg soil can be reduced by 40% compared to the control population. Nevertheless this significant reduction will not likely lead to extinction of the population as the estimated extinction time is very long; over 3000 years. Based on these results, a population of *F. candida* exposed to this concentration of Cd may not be considered endangered. However, other stresses (like habitat fragmentation, food limitation, disease, preda-

tion, etc.) can additionally influence the mean time to extinction. Similar to other population-level endpoints, one of the drawbacks of mean time to extinction as an end-point for ecological risk assessment is that there can be a significant reduction in population size, and thus ecological service, without an equally large effect on extinction time (for example due to density-dependence). Thus, from the ecological risk assessment perspective, in which the focus is on assessing effects on entire ecosystems, this single population based endpoint provides limited information to assess toxicity at the ecosystem level. One possible way to improve the potential use of the model for ERA purposes is to integrate the model with ecosystem service models or identify the role of the species in the ecological services. The stochastic density-dependent model I presented in Chapter 4 proved to be a convenient tool for extrapolating bioassay data on toxicity of chemicals to population-level effects under more realistic field conditions. It requires only a few additional pieces of information on species biology, which can be gathered relatively easily in laboratory studies. With adequate knowledge on species biology, the model can be easily adapted to virtually any species and environmental situation, and can make use of data on actual environmental conditions used for site-specific ecological risk assessment. The model for *Folsomia candida* was parameterized using published and experimental data obtained

from my own experiments on individual vital rates, and its credibility was tested against actual published data for population dynamics of the species. The model was able to estimate population-level rates with good precision for a range of tested temperatures. I therefore believe that this modeling approach can be useful for ecological risk assessment in complex and dynamic environmental situations. However, it is worth mentioning here that it would be interesting to further validate the practical applicability of the model by verifying the model simulation results based on a study of the effect of Cd at different constant and variable temperature conditions and at different laboratory population sizes.

7.6 Mechanistic interpretations of the combined effects of environmental stressors

The population model I developed in Chapter 4 was parameterized using individual-level life cycle data estimated from empirical dose–response relationships. These individual–level effect models were formulated based on observations at the end of the test by considering the external concentrations as a measure of indexes. This procedure, however, hampers the potential relevance of population modeling as a tool in ecological risk assessment (ERA) due to the lack of a mechanistic basis. Such a mechanistic basis is particularly important for

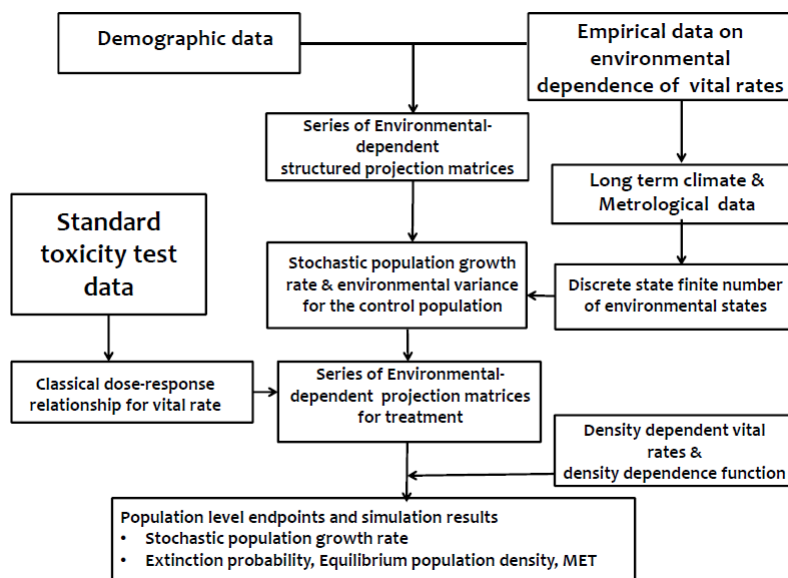


Figure 7.2: Schematic descriptions of the stochastic density dependent matrix (SDDM) model framework and its elements. The framework involves five main elements and sub-models: formulating the projection matrix, modeling effect of toxicants, estimating environmental stochasticity, modeling density-dependence and estimating overall population-level effects. The main information required for the model are: demographic data at different environmental conditions, climate data of the study area, individual-level toxicity data, and information on the effect of population density on vital rates together with the function that describe the density-dependence. MET represents mean extinction time.

the extrapolation of effects. Time is another important factor that is not usually considered in standard bioassays. To exert toxic effects, chemicals must be transported from the environment to target sites, and must accumulate there to concentrations that drive the toxic ef-

fects. The chemical-specific nature of these processes is completely ignored, as standard tests are conducted for the same specified duration for all chemicals. A final limitation of standard tests is that they focus on effects on a single endpoint only, often the endpoint that is considered to be demographically sensitive. However, as I demonstrated in Chapter 2 of this thesis, effects on the most sensitive life stages do not necessarily drive population-level effects. Population models derived from empirical dose-response based models, like the SDDM model I presented in Chapter 4, also have limitations in the mechanistic interpretation of effects. As presented in Chapter 4, the effects of the toxicant and the environmental factors are assumed to have an independent action, however, these two stressors may interact and the interaction can be more than additive or less than additive. However, the stochastic population model approaches lack any mechanistic understanding of these interactions. What is more, the method is limited to organisms whose vital rates at different environmental conditions can be accessed easily; though, this is not a problem for most of the standard species. Thus, it would be difficult to apply the technique to species with limited demographic data. On the other hand, we may also need to include multiple environmental stressors that can influence the outcome of the individual and population-level effects of toxicants. Application of stochastic models

for extrapolating these combined effects would be difficult. The limitations mentioned above can be overcome, and a reliable population model can be developed, by formulating mechanistic effect models at the individual level to interpret and predict organism-level effects of chemicals. It can also help to make an educated extrapolation to the population-level. Such mechanistic effect models should preferably be derived from a general and comprehensive energetic theory, and the Dynamic Energy Budget (DEB) theory is currently the most comprehensive and best-tested theory in this field [24–26].

In Chapter 6 of this thesis, I applied a simplified DEB model to investigate how temperature influences the effects of cadmium on vital rates of the springtail *F. candida*. Using this model, I was able to interpret the combined effects of the two stressors mechanistically, and I integrated the model with the Euler-Lotka equation to predict the effects on the population growth rate of the organism. As discussed in Section 7.2, the Euler-Lotka equation has more advantages to link with DEB-based models [13]. Figure 7.3 shows the schematic description of the general model framework used. The development and implementation of this model framework for particular species requires, however, reasonable model parameters for the organism of interest. In Chapter 5, I focused on laying out the model framework and estimating the parameters involved in the model by performing

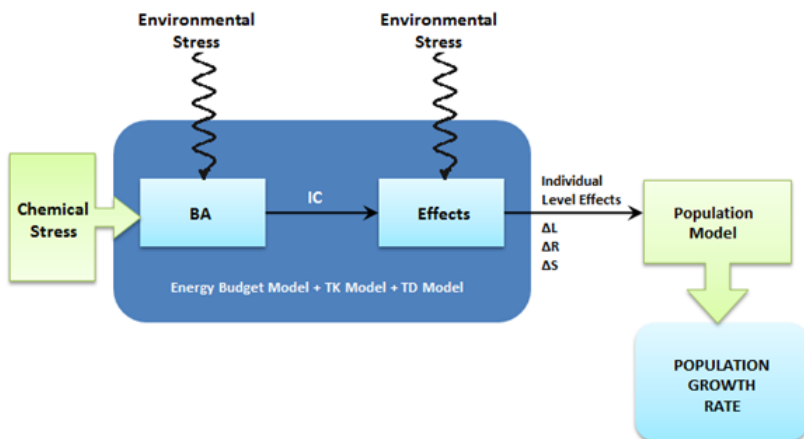


Figure 7.3: Schematic description of elements of the mechanistic model applied in the study. The model comprises three main elements: the energy budget model, the toxicokinetic (TK) model and the toxicodynamic (TD) model. The TK model determines the bioaccumulation (BA) of the toxicant, which determines the internal concentration (IC). The IC induces effects on the individual-level end points which are modelled using the TD model and expressed as change in growth (ΔL), reproduction (ΔR) and survival (ΔS). The effects of natural environmental stressors are included in the model by forcing environment-dependent TK/TD parameters. The population model then uses individual-level traits to calculate the population growth rate.

experiments, designed specifically for this purpose. Using the experimental study at three feeding regimes and three temperatures, I parameterized and calibrated the toxicant-free DEB model for *F. candida*. In the DEB context, the effects of toxicants on vital rates of an organism occur by altering one or more of the metabolic processes

in the energetic model [27]. The severity of the effect on the metabolic process is directly related to the concentration of the toxicant inside the organism. Thus, I integrated the DEB model I developed and parameterized in Chapter 5, with two additional models: a model that links external concentrations to internal concentrations (toxicokinetics, TK), and a model that links internal concentrations to effects on one or more of the parameters of the energy budget (toxicodynamics, TD). These two models also include parameters that describe the TK and TD process. In Chapter 6, I conducted a detailed cadmium toxicity study at three different temperatures covering 12 weeks of the life history of the organism and parameterized the models. Figure 7.4 shows the model development process and elements of the mechanistic effect model.

In Chapter 5, the toxicant-free simplified DEB model was applied to explore the effects of food limitation and temperature on the life history and population dynamics of *F. candida*. In this chapter, I showed how the various model parameters can be obtained by performing simple life-cycle experiments and observing standard end-points at different environmental conditions. Using the parameterized model, I was able to describe the growth and reproduction data of *F. candida* obtained from the study. However, at an early juvenile stage, the growth of *F. candida* deviated from the model. In a recent study,

Kooijman [28] discussed the different forms of metabolic acceleration that lead to this kind of deviation. The growth data for *F. candida* are well described by type X acceleration (refer to [28] for a detailed discussion). I explained this as juvenile stage food limitation and I provided an explanation for this observed deviation and suggested a model extension. At their early stage, the juveniles feed inefficiently compared to the older juveniles and adults. A possible explanation for this initial period of low feeding might be the need for development of a fully functional gut flora to aid digestion, or the need for learning of a new feeding method and adaptation to the environment. Another potential explanation for this initial slow feeding might be behavioral, which is related to the age-dependent dispersion behavior the organisms. I included this phenomenon in the model by assuming a feeding stress factor for early stage juveniles. Using the Arrhenius relationship, I was able to describe the effect of temperature on reproduction and growth data. However, at 25 °C, the model over-predicted the cumulative reproduction rate of *F. candida*. I explained this discrepancy based on the fact that 25 °C is near to the highest threshold temperature for reproduction, which is supported by previous studies [29]. I showed that the life history of *F. candida* is influenced by both food limitation and temperature in a predictable manner. Using the simulation model, I was able to extrapolate the effects of

food density and temperature on the population growth rate of *F. candida* and, further, to predict the combined effect of food density and temperature on the population growth rate. I was also able to characterize the nature of the interaction between the two environmental stressors. I showed that food limitation and temperature can have different interactions (i.e., either additive, less than additives or more than additive) on the dynamics of *F. candida*.

In Chapter 6, the toxicant-free simplified DEB model has been extended by including the TK/TD models and parameterized. The parameterized model was subsequently applied to explore the effects of temperature and time on the classic summary statistics, EC_x for growth and reproduction (the estimated concentration of Cd at which growth and reproduction were reduced to $x\%$ of that of the control). Using the population model, I derived a dose-response (or a concentration-effect) relationship between Cd and the intrinsic population growth rate of *F. candida* at different temperatures. Using this temperature dependent concentration-effect relationship, I further explored effects of the interaction between the two stressors on the population dynamics. The results suggest that temperature influences the effects of Cd on *F. candida*; which allow a mechanistic understanding of the combined effects of the two stressors. The model framework I presented in Chapter 6 is a useful tool to address an im-

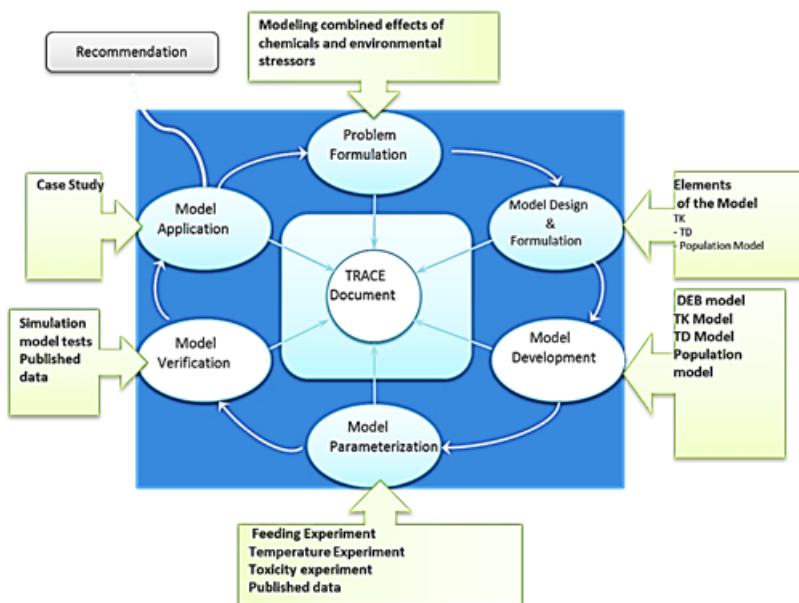


Figure 7.4: The model development process. The model development involved three main steps: the conceptual representation of the model, the functional representation of the model, and the computational representation of the model. The conceptual representation of the model involved the problem formulation, model design and model development steps. In the functional representation step of the model, the model equations which include the simplified DEB model, the TK model, the TD model and the population dynamics model were formulated. The analytical, numerical and statistical techniques together with the MATLAB program code used in the model represent the computational representation step of the model. All these processes will be documented and filed using the TRACE documentation format and can be accessed from <http://cream-itn.eu>.

portant issue in risk assessment; i.e., extrapolation of effects observed in laboratory to individuals and populations living in the field.

7.7 Concluding remarks and suggested future work

Due to the practical difficulties and resource requirements associated with assessing and predicting impacts on populations empirically, models can play a key role in population-level ecological risk assessment. This thesis shows the potential use of population models to link laboratory toxicity results to population responses. Particularly, the thesis sheds light on how to extrapolate the combined effects of toxicants and natural environmental factors and interactions of environment-specific factors and toxicants. The modelling approaches presented in this thesis can be a convenient tool to design short term toxicity tests and to extrapolate bioassay data on toxicity of chemicals to population-level effects under more realistic field conditions. However, further work is needed in investigating spatial and temporal variation of contaminant concentration in the environment. It would be also interesting to further validate the practical applicability of the models presented in the thesis by verifying the model simulation results against actual studies on effects of different types of chemicals at variable environmental conditions and at different laboratory population sizes.

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Summary

Ecological risk assessment of chemicals aims to predict adverse effects of chemicals on natural populations by comparing the predicted environmental concentration with the no-effect concentration level. The information on the ecotoxicity is derived from laboratory bioassays in which individual-level effects of toxic chemicals on survival, fecundity, or body growth rate are determined. These bioassays are standardized and usually conducted under constant environmental conditions, which neglects potential influences of all other environmental factors. What is more, the experimental conditions are presumably optimal for the test organisms. However, in their natural environment, organisms rarely experience optimal or constant conditions. On the contrary, they have to cope with either suboptimal conditions or even severe environmental stress for most of their life spans. Thus, the relevance of results of standard toxicity tests for field situations is limited, and it is also economically prohibitive or just impossible to experimentally assess all possible interactions. To fill this gap, we need analytical tools that can take individual-level data as input and calculate measures of population-level performance, by considering important ecological factors that can influence the effects of toxicants at individual and population levels. This thesis is primarily aimed at developing population models to extrapolate the combined effects of

toxicants and natural environmental stressors (particularly temperature and density-dependence) from the individual to the population. The first step in this study was to implement an age-based matrix model to infer the consequences of toxicant effects observed on individuals to population end points. The primary focus of the modeling approach was to identify the extent to which individual-level responses influence the population-level effects. The results showed that the population level interpretation of the individual level experimental results requires information on the effect of the toxicant on the life stage under study and the demographic sensitivity of the population multiplication factor (λ) to changes in particular life stages. The demographic decomposition analysis presented in this part of the thesis was recommended as a tool to design short term experimental studies. In the rest of the thesis, the combined effects of natural environmental factors and cadmium on *Folsomia candida* was studied by applying different modelling approaches. *F. candida* is the most studied collembolan in ecology and ecotoxicology. Currently, the species is used as standard test organism for terrestrial invertebrates both in OECD and ISO guidelines. Initially, a stochastic density-dependence matrix model was developed and applied to investigate the combined effects of cadmium, seasonal temperature fluctuation and density-dependence on the population dynamics of *F. candida*. It was shown

that extrapolating individual-level effects of Cd to a higher level of biological organization without considering environmental variability underestimates effects of chemicals on the population growth rate. The study result showed that chemicals can also reduce the population size at its carrying capacity. The latter phenomenon arises from different effects of toxicants on different age/stage classes combined with natural demographic processes. Mean time to extinction (MTE) was applied as population-level index to combine all these effects (i.e., environmental variability, chemical-specific toxicity, and density dependence). From the model simulation, it was shown that the mean time to extinction of a *F. candida* population exposed to 1000 mg Cd/kg soil can be reduced by 40% compared to the control population. Nevertheless, this significant reduction will not lead to rapid extinction of the population as the estimated mean time to extinction is still over 3000 years. Based on these results, a population of *F. candida* exposed to this concentration of Cd may not be considered to be endangered. The population-level effects assessment presented in this study is one possible improvement in risk assessment, and an extension of the methodology applied, would be to add yet another end-point which can be calculated using the approach presented herein, namely the equilibrium size of a population under pollution pressure.

A more generic and mechanistic model was developed by linking an energetic based individual-level effect model with the Euler-Lotka equation. Formulating mechanistic effect models to interpret organism-level effects of chemicals helps to understand the interactions between individuals and the environment. It can also help to make an educated extrapolation to the population-level. Such mechanistic effect models can be derived from a general and comprehensive energetic theory: the Dynamic Energy Budget (DEB) theory. DEB theory provides a mechanistic interpretation of how organisms acquire and use energy. The development and implementation of this model framework for particular species requires, however, reasonable model parameters for the organism of interest. Thus, as first step a suitable experiment was designed using a simplified DEB model (“DEBkiss”), which was parameterized and calibrated for *F. candida*. The DEBkiss model successfully captured the growth and reproduction trends of the organism at different feeding and temperature conditions, but at the early juvenile stage, the growth of *F. candida* deviated from the von Bertalanffy curve. We assumed an age-dependent switch in feeding behavior of the juveniles, which resulted in a good fit to the body size data for all observed data sets. The model was linked with the Euler-Lotka equation to predict the combined effect of food density and temperature on the population growth rate of *F. candida*. We

conclude that the life history of *F. candida* is influenced by both food limitation and temperature in a predictable manner. We also showed that food limitation and temperature can have different forms of interactions on the dynamics of *F. candida*. In addition to this ecological application, the potential use of the model framework in the ERA process was presented. For this we applied the DEBkiss model to capture the combined effects of Cd and temperature on the growth and reproduction of *F. candida*, and then we applied the Euler-Lotka equation to extrapolate these individual-level effects to population-level. At the individual-level, the DEBkiss model was able to capture the effects of Cd on *F. candida* at 15 and 20 °C, but due to the higher mortality and low reproduction, we could not make reliable fit at 25 °C. Using the model outputs, we estimated EC₂₅ and EC₅₀ for growth and reproduction, respectively, at both at 15 and 20 °C. These estimates were lower at 15 °C, which indicates that the magnitude of the effects on vital rates of *F. candida* is higher at 15 °C than 20 °C. The response of *F. candida* population to increasing concentrations of Cd was predicted using the Euler-Lotka equation. We found that the population growth rate of *F. candida* decreases at a higher rate at 15 °C than at 20 °C with increased cadmium concentrations. We conclude that extrapolation of effects from the current 20 °C based standard toxicity tests to individuals and population living in different

temperature conditions might lead to erroneous conclusion. We show the great potential of energetic-based models, like DEBkiss, for the mechanistic interpretation of the combined effects of multiple stressors on individuals; and their advantages for developing population models to extrapolation effects to population-level. In recent time, a tendency has developed to integrate population models in risk assessment process, thus the model framework presented in this study can be one potential tool to address the objectives of this new paradigm in ERA.

Samenvatting

Ecologische risicobeoordeling is gericht op het voorspellen van nadelige effecten van stoffen op natuurlijke populaties. In het algemeen wordt hierbij de voorspelde blootstelling aan de stof vergeleken met een voorspelde veilige concentratie. De informatie over de toxiciteit wordt verkregen uit toxiciteitstesten waarin de toxische effecten op individuniveau bepaald worden. Hierbij wordt gekeken naar eigenschappen zoals overleving, vruchtbaarheid en lichaamsgroei. Deze testen zijn gestandaardiseerd, en worden gewoonlijk uitgevoerd onder constante laboratoriumomstandigheden. Hierdoor wordt voorbijgegaan aan de potentiële invloed van milieufactoren op de testresultaten. In de test wordt de omgeving zo gekozen dat deze (waarschijnlijk) optimaal is voor de diersoort. In hun natuurlijke omgeving zijn de omstandigheden echter zelden of nooit optimaal voor het organisme. In tegendeel: organismen zullen regelmatig geconfronteerd worden met niet-optimale omstandigheden en zelfs met zware stress. De relevantie van de standaard toxiciteitstesten voor de situatie in het veld is dus gering, en het is duur of zelfs onmogelijk om alle mogelijke interacties experimenteel te testen. Om dit gat te vullen zijn analysetechnieken nodig die in staat zijn om effecten op individuniveau te vertalen naar een maat voor de gezondheid van de populatie, rekening houdend met belangrijke milieufactoren die de effecten van toxicanten kunnen

beïnvloeden.

Dit proefschrift is gericht op het ontwikkelen van populatiemodellen voor het extrapoleren van het gecombineerde effect van toxicanten en milieufactoren (met name temperatuur en populatiedichtheid) van individu naar populatie. De eerste stap in deze studie was het implementeren van een leeftijd-gebaseerd matrixmodel om de populatie-effecten af te kunnen leiden. Deze modelaanpak was met name gericht op het onderzoeken van de mate waarin effecten op individuen leiden tot effecten op de populatie. De resultaten lieten zien dat hiervoor zowel informatie over het effect van de toxicant op verschillende levensstadia nodig is, alsmede de demografische gevoeligheid van de populatiegroei voor veranderingen in bepaalde levensstadia. De decompositiemethode die ik hiervoor gebruikt heb is een bruikbare hulp bij het ontwerpen van korte toxiciteitsstudies.

In de rest van mijn proefschrift behandel ik het gezamenlijke effect van milieufactoren en cadmium op de springstaart *Folsomia candida* met behulp van verschillende modelaanpakken. Deze soort is erg populair in ecologische en ecotoxicologische studies, en is een standaard testorganisme in risicobeoordeling voor de bodem. Ik ben begonnen met het ontwikkelen van een stochastisch matrixmodel, rekening houdend met dichtheidsafhankelijke effecten, om het gecombineerde effect van cadmium, seizoensgebonden temperatuursfluctu-

aties, en populatiedichtheid te bestuderen. Het niet meenemen van milieufactoren bleek te leiden tot een onderschatting van het effect van cadmium op de populatiegroei. Verder bleek dat stoffen ook de maximale populatieomvang kunnen reduceren, als resultaat van het gecombineerde effect van toxicanten op de diverse leeftijdsklassen en de natuurlijke demografische processen. De gemiddelde tijd tot uitsterven van de populatie werd gebruikt als een index voor de gecombineerde effecten (milieufunctuaties, toxiciteit en dichtheidseffecten). Modelsimulaties lieten zien dat de gemiddelde tijd tot uitsterven voor een populatie springstaarten blootgesteld aan 1000 mg cadmium per kilo grond zo'n 40% gereduceerd kan zijn ten opzichte van de controle. Deze reductie blijkt echter niet tot uitsterven van de populatie te leiden; de gemiddelde tijd tot uitsterven was meer dan 3000 jaar. Op basis van deze resultaten kan men deze concentratie cadmium als "veilig" beschouwen voor een populatie van deze soort. Een effectbeoordeling op populatieniveau, zoals gepresenteerd in dit proefschrift, is een mogelijke verbetering voor de risicobeoordeling van toxicanten. Als uitbreiding kan verder nog gedacht worden aan het rapporteren van een andere modeluitkomst, namelijk de stabiele populatieomvang onder toxische druk.

Als volgende stap in deze studie heb ik een meer generiek mechanistisch model ontwikkeld door het koppelen van een energetisch model

voor individuen aan een populatieberekening middels de Euler-Lotka-vergelijking. Het formuleren van een mechanistisch model op het individuniveau helpt om de interacties tussen individu en omgeving te begrijpen, en is onontbeerlijk voor een onderbouwde extrapolatie naar het populatieniveau. Zulke modellen voor individuen kunnen worden afgeleid van de theorie voor Dynamische EnergieBudgetten (DEB). De DEB-theorie geeft een mechanistische interpretatie van het energetische huishoudboekje van een organisme. Het ontwikkelen en implementeren van dit modelkader voor een specifieke soort vereist redelijke waarden voor de modelparameters. Daarom heb ik een toepasselijk experiment ontworpen, gebruik makend van een versimpeld DEB-model (“DEBkiss”). Het model werd middels de uitkomsten van het experiment geparameteriseerd voor *F. candida*. Het DEBkiss-model was in staat om de patronen in groei en reproductie te verklaren bij verschillende voedselniveaus en verschillende temperaturen. De pas uitgekomen dieren lieten echter een afwijking van het voorspelde groeipatroon zien. Hiervoor neem ik een leeftijdsafhankelijke overgang in het eetgedrag aan, wat leidt tot een goede verklaring voor de groei in alle behandelingen. Het DEBkiss-model werd gekoppeld aan de Euler-Lotka-vergelijking om het gecombineerde effect van voedselaanbod en temperatuur op de populatiegroei te voorspellen. Het is duidelijk dat de levenscyclus van *F. candida* op een voorspel-

bare manier beïnvloed wordt door zowel voedsel als temperatuur. Ik laat ook zien dat voedselgebrek en temperatuur verschillende vormen van interactie vertonen op de populatiegroei.

Na deze ecologische toepassing van het model heb ik vervolgens gekeken naar de mogelijkheden voor toepassing in ecologische risicobeoordeling voor toxicanten. Hiertoe gebruikte ik het DEBkiss-model om de gecombineerde effecten van cadmium en temperatuur op de groei en reproductie van *F. candida* te verklaren, en de Euler-Lotka-vergelijking om de effecten van individu naar populatie te vertalen. Op individuniveau was het DEBkiss-model in staat om de effecten van cadmium te verklaren bij 15 en 20°C, maar niet bij 25°C door een hoge sterfte en erg lage reproductie. De modeluitkomsten laten zien dat de er meer effect van cadmium is op groei en reproductie bij 15°C dan bij 20°C. Verder neemt de populatiegroei (bepaald met de Euler-Lotka-vergelijking) bij 15°C sneller af met cadmiumblootstelling dan bij 20°C. Omdat de huidige standaardtesten worden uitgevoerd bij 20°C kan dit leiden tot verkeerde conclusies met betrekking tot de toxiciteit van stoffen. Deze studie laat duidelijk de enorme mogelijkheden van energetische modellen zoals DEBkiss zien bij het interpreteren van de gecombineerde effecten van verschillende stressoren op individuen. Deze aanpak voor individuen biedt ook grote voordelen voor het ontwikkelen van populatiemodellen voor de vertaling van individu naar

populatie-effecten. Er is momenteel een ontwikkeling gaande om populatiemodellen in de risicobeoordeling te integreren. De modelkaders gepresenteerd in dit proefschrift vormen een potentiële bijdrage aan dit nieuwe paradigma.